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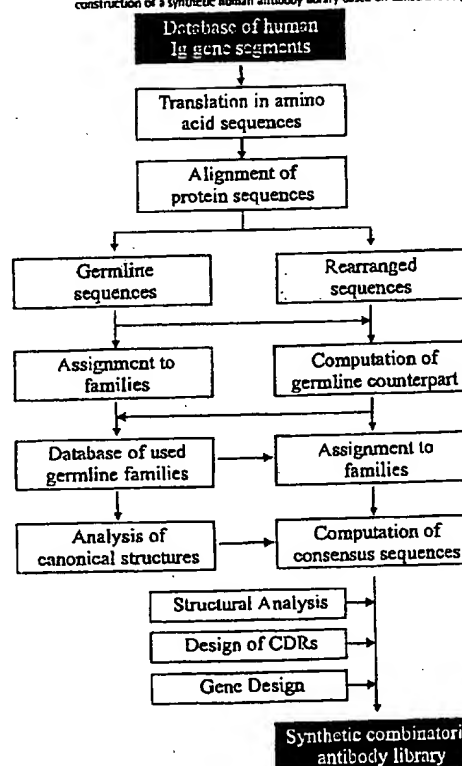
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(54) Title: PROTEIN/(POLY)PEPTIDE LIBRARIES

(57) Abstract

The present invention relates to synthetic DNA sequences which encode one or more collections of homologous proteins/(poly)peptides, and methods for generating and applying libraries of these DNA sequences. In particular, the invention relates to the preparation of a library of human-derived antibody genes by the use of synthetic consensus sequences which cover the structural repertoire of antibodies encoded in the human genome. Furthermore, the invention relates to the use of a single consensus antibody gene as a universal framework for highly diverse antibody libraries.

construction of a synthetic human antibody library based on consensus sequences



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## Protein/(Poly)peptide Libraries

### Field of the Invention

The present invention relates to synthetic DNA sequences which encode one or more collections of homologous proteins/(poly)peptides, and methods for generating and applying libraries of these DNA sequences. In particular, the invention relates to the preparation of a library of human-derived antibody genes by the use of synthetic consensus sequences which cover the structural repertoire of antibodies encoded in the human genome. Furthermore, the invention relates to the use of a single consensus antibody gene as a universal framework for highly diverse antibody libraries.

### Background to the Invention

All current recombinant methods which use libraries of proteins/(poly)peptides, e.g. antibodies, to screen for members with desired properties, e.g. binding a given ligand, do not provide the possibility to improve the desired properties of the members in an easy and rapid manner. Usually a library is created either by inserting a random oligonucleotide sequence into one or more DNA sequences cloned from an organism, or a family of DNA sequences is cloned and used as the library. The library is then screened, e.g. using phage display, for members which show the desired property. The sequences of one or more of these resulting molecules are then determined. There is no general procedure available to improve these molecules further on.

Winter (EP 0 368 684 B1) has provided a method for amplifying (by PCR), cloning, and expressing antibody variable region genes. Starting with these genes he was able to create libraries of functional antibody fragments by randomizing the CDR3 of the heavy and/or the light chain. This process is functionally equivalent to the natural process of VJ and VDJ recombination which occurs during the development of B-cells in the immune system.

However the Winter invention does not provide a method for optimizing the binding affinities of antibody fragments further on, a process which would be functionally equivalent to the naturally occurring phenomenon of "affinity maturation", which is provided by the present invention. Furthermore, the Winter invention does not provide for artificial variable region genes, which represent a whole family of

structurally similar natural genes, and which can be assembled from synthetic DNA oligonucleotides. Additionally, Winter does not enable the combinatorial assembly of portions of antibody variable regions, a feature which is provided by the present invention. Furthermore, this approach has the disadvantage that the genes of all antibodies obtained in the screening procedure have to be completely sequenced, since, except for the PCR priming regions, no additional sequence information about the library members is available. This is time and labor intensive and potentially leads to sequencing errors.

The teaching of Winter as well as other approaches have tried to create large antibody libraries having high diversity in the complementarity determining regions (CDRs) as well as in the frameworks to be able to find antibodies against as many different antigens as possible. It has been suggested that a single universal framework may be useful to build antibody libraries, but no approach has yet been successful.

Another problem lies in the production of reagents derived from antibodies. Small antibody fragments show exciting promise for use as therapeutic agents, diagnostic reagents, and for biochemical research. Thus, they are needed in large amounts, and the expression of antibody fragments, e.g. Fv, single-chain Fv (scFv), or Fab in the periplasm of *E. coli* (Skerra & Plückthun, 1988; Better et al., 1988) is now used routinely in many laboratories. Expression yields vary widely, however. While some fragments yield up to several mg of functional, soluble protein per liter and OD of culture broth in shake flask culture (Carter et al., 1992, Plückthun et al. 1996), other fragments may almost exclusively lead to insoluble material, often found in so-called inclusion bodies. Functional protein may be obtained from the latter in modest yields by a laborious and time-consuming refolding process. The factors influencing antibody expression levels are still only poorly understood. Folding efficiency and stability of the antibody fragments, protease lability and toxicity of the expressed proteins to the host cells often severely limit actual production levels, and several attempts have been tried to increase expression yields. For example, Knappik & Plückthun (1995) could show that expression yield depends on the antibody sequence. They identified key residues in the antibody framework which influence expression yields dramatically. Similarly, Ullrich et al. (1995) found that point mutations in the CDRs can increase the yields in periplasmic antibody fragment expression. Nevertheless, these strategies are only applicable to a few antibodies. Since the Winter invention uses existing repertoires of antibodies, no influence on expressibility of the genes is possible.



Furthermore, the findings of Knappik & Plückthun and Ullrich demonstrate that the knowledge about antibodies, especially about folding and expression is still increasing. The Winter invention does not allow to incorporate such improvements into the library design.

The expressibility of the genes is important for the library quality as well, since the screening procedure relies in most cases on the display of the gene product on a phage surface, and efficient display relies on at least moderate expression of the gene.

These disadvantages of the existing methodologies are overcome by the present invention, which is applicable for all collections of homologous proteins. It has the following novel and useful features illustrated in the following by antibodies as an example:

Artificial antibodies and fragments thereof can be constructed based on known antibody sequences, which reflect the structural properties of a whole group of homologous antibody genes. Therefore it is possible to reduce the number of different genes without any loss in the structural repertoire. This approach leads to a limited set of artificial genes, which can be synthesized *de novo*, thereby allowing introduction of cleavage sites and removing unwanted cleavage sites. Furthermore, this approach enables (i), adapting the codon usage of the genes to that of highly expressed genes in any desired host cell and (ii), analyzing all possible pairs of antibody light (L) and heavy (H) chains in terms of interaction preference, antigen preference or recombinant expression titer, which is virtually impossible using the complete collection of antibody genes of an organism and all combinations thereof.

The use of a limited set of completely synthetic genes makes it possible to create cleavage sites at the boundaries of encoded structural sub-elements. Therefore, each gene is built up from modules which represent structural sub-elements on the protein/(poly)peptide level. In the case of antibodies, the modules consist of "framework" and "CDR" modules. By creating separate framework and CDR modules, different combinatorial assembly possibilities are enabled. Moreover, if two or more artificial genes carry identical pairs of cleavage sites at the boundaries of each of the genetic sub-elements, pre-built libraries of sub-elements can be inserted in these genes simultaneously, without any additional information related to any particular gene sequence. This strategy enables rapid optimization of, for example, antibody affinity, since DNA cassettes encoding libraries of genetic sub-elements can be (i), pre-built, stored and reused and (ii), inserted in any of these

sequences at the right position without knowing the actual sequence or having to determine the sequence of the individual library member.

Additionally, new information about amino acid residues important for binding, stability, or solubility and expression could be integrated into the library design by replacing existing modules with modules modified according to the new observations.

The limited number of consensus sequences used for creating the library allows to speed up the identification of binding antibodies after screening. After having identified the underlying consensus gene sequence, which could be done by sequencing or by using fingerprint restriction sites, just those part(s) comprising the random sequence(s) have to be determined. This reduces the probability of sequencing errors and of false-positive results.

The above mentioned cleavage sites can be used only if they are unique in the vector system where the artificial genes have been inserted. As a result, the vector has to be modified to contain none of these cleavage sites. The construction of a vector consisting of basic elements like resistance gene and origin of replication, where cleavage sites have been removed, is of general interest for many cloning attempts. Additionally, these vector(s) could be part of a kit comprising the above mentioned artificial genes and pre-built libraries.

The collection of artificial genes can be used for a rapid humanization procedure of non-human antibodies, preferably of rodent antibodies. First, the amino acid sequence of the non-human, preferably rodent antibody is compared with the amino acid sequences encoded by the collection of artificial genes to determine the most homologous light and heavy framework regions. These genes are then used for insertion of the genetic sub-elements encoding the CDRs of the non-human, preferably rodent antibody.

Surprisingly, it has been found that with a combination of only one consensus sequence for each of the light and heavy chains of a scFv fragment an antibody repertoire could be created yielding antibodies against virtually every antigen. Therefore, one aspect of the present invention is the use of a single consensus sequence as a universal framework for the creation of useful (poly)peptide libraries and antibody consensus sequences useful therefor.

## Detailed Description of the Invention

The present invention enables the creation of useful libraries of (poly)peptides. In a first embodiment, the invention provides for a method of setting up nucleic acid sequences suitable for the creation of said libraries. In a first step, a collection of at least three homologous proteins is identified and then analyzed. Therefore, a database of the protein sequences is established where the protein sequences are aligned to each other. The database is used to define subgroups of protein sequences which show a high degree of similarity in both the sequence and, if information is available, in the structural arrangement. For each of the subgroups a (poly)peptide sequence comprising at least one consensus sequence is deduced which represents the members of this subgroup; the complete collection of (poly)peptide sequences represent therefore the complete structural repertoire of the collection of homologous proteins. These artificial (poly)peptide sequences are then analyzed, if possible, according to their structural properties to identify unfavorable interactions between amino acids within said (poly)peptide sequences or between said or other (poly)peptide sequences, for example, in multimeric proteins. Such interactions are then removed by changing the consensus sequence accordingly. The (poly)peptide sequences are then analyzed to identify sub-elements such as domains, loops, helices or CDRs. The amino acid sequence is backtranslated into a corresponding coding nucleic acid sequence which is adapted to the codon usage of the host planned for expressing said nucleic acid sequences. A set of cleavage sites is set up in a way that each of the sub-sequences encoding the sub-elements identified as described above, is flanked by two sites which do not occur a second time within the nucleic acid sequence. This can be achieved by either identifying a cleavage site already flanking a sub-sequence or by changing one or more nucleotides to create the cleavage site, and by removing that site from the remaining part of the gene. The cleavage sites should be common to all corresponding sub-elements or sub-sequences, thus creating a fully modular arrangement of the sub-sequences in the nucleic acid sequence and of the sub-elements in the corresponding (poly)peptide.

In a further embodiment, the invention provides for a method which sets up two or more sets of (poly)peptides, where for each set the method as described above is performed, and where the cleavage sites are not only unique within each set but also between any two sets. This method can be applied for the creation of (poly)peptide libraries comprising for example two  $\alpha$ -helical domains from two different proteins, where said library is screened for novel hetero-association domains.

In yet a further embodiment, at least two of the sets as described above, are derived from the same collection of proteins or at least a part of it. This describes libraries comprising for example, but not limited to, two domains from antibodies such as VH and VL, or two extracellular loops of transmembrane receptors.

In another embodiment, the nucleic acid sequences set up as described above, are synthesized. This can be achieved by any one of several methods well known to the practitioner skilled in the art, for example, by total gene synthesis or by PCR-based approaches.

In one embodiment, the nucleic acid sequences are cloned into a vector. The vector could be a sequencing vector, an expression vector or a display (e.g. phage display) vector, which are well known to those skilled in the art. Any vector could comprise one nucleic acid sequence, or two or more nucleic sequences, either in different or the same operon. In the last case, they could either be cloned separately or as contiguous sequences.

In one embodiment, the removal of unfavorable interactions as described above, leads to enhanced expression of the modified (poly)peptides.

In a preferred embodiment, one or more sub-sequences of the nucleic acid sequences are replaced by different sequences. This can be achieved by excising the sub-sequences using the conditions suitable for cleaving the cleavage sites adjacent to or at the end of the sub-sequence, for example, by using a restriction enzyme at the corresponding restriction site under the conditions well known to those skilled in the art, and replacing the sub-sequence by a different sequence compatible with the cleaved nucleic acid sequence. In a further preferred embodiment, the different sequences replacing the initial sub-sequence(s) are genomic or rearranged genomic sequences, for example in grafting CDRs from non-human antibodies onto consensus antibody sequences for rapid humanization of non-human antibodies. In the most preferred embodiment, the different sequences are random sequences, thus replacing the sub-sequence by a collection of sequences to introduce variability and to create a library. The random sequences can be assembled in various ways, for example by using a mixture of mononucleotides or preferably a mixture of trinucleotides (Virnekäs et al., 1994) during automated oligonucleotide synthesis, by error-prone PCR or by other methods well known to the practitioner in the art. The random sequences may be completely randomized or biased towards or against certain codons according to

the amino acid distribution at certain positions in known protein sequences. Additionally, the collection of random sub-sequences may comprise different numbers of codons, giving rise to a collection of sub-elements having different lengths.

In another embodiment, the invention provides for the expression of the nucleic acid sequences from a suitable vector and under suitable conditions well known to those skilled in the art.

In a further preferred embodiment, the (poly)peptides expressed from said nucleic acid sequences are screened and, optionally, optimized. Screening may be performed by using one of the methods well known to the practitioner in the art, such as phage-display, selectively infective phage, polysome technology to screen for binding, assay systems for enzymatic activity or protein stability. (Poly)peptides having the desired property can be identified by sequencing of the corresponding nucleic acid sequence or by amino acid sequencing or mass spectrometry. In the case of subsequent optimization, the nucleic acid sequences encoding the initially selected (poly)peptides can optionally be used without sequencing. Optimization is performed by repeating the replacement of sub-sequences by different sequences, preferably by random sequences, and the screening step one or more times.

The desired property the (poly)peptides are screened for is preferably, but not exclusively, selected from the group of optimized affinity or specificity for a target molecule, optimized enzymatic activity, optimized expression yields, optimized stability and optimized solubility.

In one embodiment, the cleavage sites flanking the sub-sequences are sites recognized and cleaved by restriction enzymes, with recognition and cleavage sequences being either identical or different, the restricted sites either having blunt or sticky ends.

The length of the sub-elements is preferably, but not exclusively ranging between 1 amino acid, such as one residue in the active site of an enzyme or a structure-determining residue, and 150 amino acids, as for whole protein domains. Most preferably, the length ranges between 3 and 25 amino acids, such as most commonly found in CDR loops of antibodies.

The nucleic acid sequences could be RNA or, preferably, DNA.

In one embodiment, the (poly)peptides have an amino acid pattern characteristic of a particular species. This can for example be achieved by deducing the consensus sequences from a collection of homologous proteins of just one species, most preferably from a collection of human proteins. Since the (poly)peptides comprising consensus sequences are artificial, they have to be compared to the protein sequence(s) having the closest similarity to ensure the presence of said characteristic amino acid pattern.

In one embodiment, the invention provides for the creation of libraries of (poly)peptides comprising at least part of members or derivatives of the immunoglobulin superfamily, preferably of member or derivatives of the immunoglobulins. Most preferably, the invention provides for the creation of libraries of human antibodies, wherein said (poly)peptides are or are derived from heavy or light chain variable regions wherein said structural sub-elements are framework regions (FR) 1, 2, 3, or 4 or complementary determining regions (CDR) 1, 2, or 3. In a first step, a database of published antibody sequences of human origin is established where the antibody sequences are aligned to each other. The database is used to define subgroups of antibody sequences which show a high degree of similarity in both the sequence and the canonical fold of CDR loops (as determined by analysis of antibody structures). For each of the subgroups a consensus sequence is deduced which represents the members of this subgroup; the complete collection of consensus sequences represent therefore the complete structural repertoire of human antibodies.

These artificial genes are then constructed e.g. by total gene synthesis or by the use of synthetic genetic subunits. These genetic subunits correspond to structural sub-elements on the (poly)peptide level. On the DNA level, these genetic subunits are defined by cleavage sites at the start and the end of each of the sub-elements, which are unique in the vector system. All genes which are members of the collection of consensus sequences are constructed such that they contain a similar pattern of corresponding genetic sub-sequences. Most preferably, said (poly)peptides are or are derived from the HuCAL consensus genes: V $\kappa$ 1, V $\kappa$ 2, V $\kappa$ 3, V $\kappa$ 4, V $\lambda$ 1, V $\lambda$ 2, V $\lambda$ 3, VH1A, VH1B, VH2, VH3, VH4, VH5, VH6, C $\kappa$ , C $\lambda$ , CH1 or any combination of said HuCAL consensus genes.

This collection of DNA molecules can then be used to create libraries of antibodies or antibody fragments, preferably Fv, disulphide-linked Fv, single-chain Fv (scFv), or Fab fragments, which may be used as sources of specificities against new target antigens. Moreover, the affinity of the antibodies can be optimized using pre-built library cassettes and a general procedure. The invention provides a method for identifying one or more genes encoding one or more antibody fragments which

binds to a target, comprising the steps of expressing the antibody fragments, and then screening them to isolate one or more antibody fragments which bind to a given target molecule. Preferably, an scFv fragment library comprising the combination of HuCAL VH3 and HuCAL VL2 consensus genes and at least a random sub-sequence encoding the heavy chain CDR3 sub-element is screened for binding antibodies. If necessary, the modular design of the genes can then be used to excise from the genes encoding the antibody fragments one or more genetic sub-sequences encoding structural sub-elements, and replacing them by one or more second sub-sequences encoding structural sub-elements. The expression and screening steps can then be repeated until an antibody having the desired affinity is generated.

Particularly preferred is a method in which one or more of the genetic subunits (e.g. the CDRs) are replaced by a random collection of sequences (the library) using the said cleavage sites. Since these cleavage sites are (i) unique in the vector system and (ii) common to all consensus genes, the same (pre-built) library can be inserted into all artificial antibody genes. The resulting library is then screened against any chosen antigen. Binding antibodies are selected, collected and used as starting material for the next library. Here, one or more of the remaining genetic subunits are randomized as described above.

A further embodiment of the present invention relates to fusion proteins by providing for a DNA sequence which encodes both the (poly)peptide, as described above, as well as an additional moiety. Particularly preferred are moieties which have a useful therapeutic function. For example, the additional moiety may be a toxin molecule which is able to kill cells (Vitetta et al., 1993). There are numerous examples of such toxins, well known to the one skilled in the art, such as the bacterial toxins *Pseudomonas* exotoxin A, and diphtheria toxin, as well as the plant toxins ricin, abrin, modeccin, saporin, and gelonin. By fusing such a toxin for example to an antibody fragment, the toxin can be targeted to, for example, diseased cells, and thereby have a beneficial therapeutic effect. Alternatively, the additional moiety may be a cytokine, such as IL-2 (Rosenberg & Lotze, 1986), which has a particular effect (in this case a T-cell proliferative effect) on a family of cells. In a further embodiment, the additional moiety may confer on its (poly)peptide partner a means of detection and/or purification. For example, the fusion protein could comprise the modified antibody fragment and an enzyme commonly used for detection purposes, such as alkaline phosphatase (Blake et al., 1984). There are numerous other moieties which can be used as detection or purification tags, which are well known to the practitioner skilled in the art. Particularly preferred are peptides comprising at least five histidine residues (Hochuli et al., 1988), which are able to bind to metal ions,

and can therefore be used for the purification of the protein to which they are fused (Lindner et al., 1992). Also provided for by the invention are additional moieties such as the commonly used C-myc and FLAG tags (Hopp et al., 1988; Knappik & Plückthun, 1994).

By engineering one or more fused additional domains, antibody fragments or any other (poly)peptide can be assembled into larger molecules which also fall under the scope of the present invention. For example, mini-antibodies (Pack, 1994) are dimers comprising two antibody fragments, each fused to a self-associating dimerization domain. Dimerization domains which are particularly preferred include those derived from a leucine zipper (Pack & Plückthun, 1992) or helix-turn-helix motif (Pack et al., 1993).

All of the above embodiments of the present invention can be effected using standard techniques of molecular biology known to anyone skilled in the art.

In a further embodiment, the random collection of sub-sequences (the library) is inserted into a singular nucleic acid sequence encoding one (poly)peptide, thus creating a (poly)peptide library based on one universal framework. Preferably a random collection of CDR sub-sequences is inserted into a universal antibody framework, for example into the HuCAL H3κ2 single-chain Fv fragment described above.

In further embodiments, the invention provides for nucleic acid sequence(s), vector(s) containing the nucleic acid sequence(s), host cell(s) containing the vector(s), and (poly)peptides, obtainable according to the methods described above.

In a further preferred embodiment, the invention provides for modular vector systems being compatible with the modular nucleic acid sequences encoding the (poly)peptides. The modules of the vectors are flanked by restriction sites unique within the vector system and essentially unique with respect to the restriction sites incorporated into the nucleic acid sequences encoding the (poly)peptides, except for example the restriction sites necessary for cloning the nucleic acid sequences into the vector. The list of vector modules comprises origins of single-stranded replication, origins of double-stranded replication for high- and low copy number plasmids, promotor/operator, repressor or terminator elements, resistance genes, potential recombination sites, gene III for display on filamentous phages, signal sequences, purification and detection tags, and sequences of additional moieties.



The vectors are preferably, but not exclusively, expression vectors or vectors suitable for expression and screening of libraries.

In another embodiment, the invention provides for a kit, comprising one or more of the list of nucleic acid sequence(s), recombinant vector(s), (poly)peptide(s), and vector(s) according to the methods described above, and suitable host cell(s) for producing the (poly)peptide(s).

In a preferred embodiment, the invention provides for the creation of libraries of human antibodies. In a first step, a database of published antibody sequences of human origin is established. The database is used to define subgroups of antibody sequences which show a high degree of similarity in both the sequence and the canonical fold (as determined by analysis of antibody structures). For each of the subgroups a consensus sequence is deduced which represents the members of this subgroup; the complete collection of consensus sequences represent therefore the complete structural repertoire of human antibodies.

These artificial genes are then constructed by the use of synthetic genetic subunits. These genetic subunits correspond to structural sub-elements on the protein level. On the DNA level, these genetic subunits are defined by cleavage sites at the start and the end of each of the subelements, which are unique in the vector system. All genes which are members of the collection of consensus sequences are constructed such that they contain a similar pattern of said genetic subunits.

This collection of DNA molecules can then be used to create libraries of antibodies which may be used as sources of specificities against new target antigens. Moreover, the affinity of the antibodies can be optimised using pre-built library cassettes and a general procedure. The invention provides a method for identifying one or more genes encoding one or more antibody fragments which binds to a target, comprising the steps of expressing the antibody fragments, and then screening them to isolate one or more antibody fragments which bind to a given target molecule. If necessary, the modular design of the genes can then be used to excise from the genes encoding the antibody fragments one or more genetic sub-sequences encoding structural sub-elements, and replacing them by one or more second sub-sequences encoding structural sub-elements. The expression and screening steps can then be repeated until an antibody having the desired affinity is generated.

Particularly preferred is a method in which one or more of the genetic subunits (e.g. the CDR's) are replaced by a random collection of sequences (the library) using the said cleavage sites. Since these cleavage sites are (i) unique in the vector system and (ii) common to all consensus genes, the same (pre-built) library can be inserted into all artificial antibody genes. The resulting library is then screened against any chosen antigen. Binding antibodies are eluted, collected and used as starting material for the next library. Here, one or more of the remaining genetic subunits are randomised as described above.

## Definitions

### Protein:

The term protein comprises monomeric polypeptide chains as well as homo- or heteromultimeric complexes of two or more polypeptide chains connected either by covalent interactions (such as disulphide bonds) or by non-covalent interactions (such as hydrophobic or electrostatic interactions).

### Analysis of homologous proteins:

The amino acid sequences of three or more proteins are aligned to each other (allowing for introduction of gaps) in a way which maximizes the correspondence between identical or similar amino acid residues at all positions. These aligned sequences are termed homologous if the percentage of the sum of identical and/or similar residues exceeds a defined threshold. This threshold is commonly regarded by those skilled in the art as being exceeded when at least 15% of the amino acids in the aligned genes are identical, and at least 30% are similar. Examples for families of homologous proteins are: immunoglobulin superfamily, scavenger receptor superfamily, fibronectin superfamilies (e.g. type II and III), complement control protein superfamily, cytokine receptor superfamily, cystine knot proteins, tyrosine kinases, and numerous other examples well known to one of ordinary skill in the art.

### Consensus sequence:

Using a matrix of at least three aligned amino acid sequences, and allowing for gaps in the alignment, it is possible to determine the most frequent amino acid residue at each position. The consensus sequence is that sequence which comprises the amino acids which are most frequently represented at each position. In the event that two or more amino acids are equally represented at a single position, the consensus sequence includes both or all of those amino acids.

### Removing unfavorable interactions:

The consensus sequence is per se in most cases artificial and has to be analyzed in order to change amino acid residues which, for example, would prevent the resulting molecule to adapt a functional tertiary structure or which would block the interaction with other (poly)peptide chains in multimeric complexes. This can be done either by (i) building a three-dimensional model of the consensus sequence using known related structures as a template, and identifying amino acid residues within the model which may interact unfavorably with each other, or (ii) analyzing the matrix of aligned amino acid sequences in order to detect combinations of amino

acid residues within the sequences which frequently occur together in one sequence and are therefore likely to interact with each other. These probable interaction-pairs are then tabulated and the consensus is compared with these "interaction maps". Missing or wrong interactions in the consensus are repaired accordingly by introducing appropriate changes in amino acids which minimize unfavorable interactions.

Identification of structural sub-elements:

Structural sub-elements are stretches of amino acid residues within a protein/(poly)peptide which correspond to a defined structural or functional part of the molecule. These can be loops (e.g. CDR loops of an antibody) or any other secondary or functional structure within the protein/(poly)peptide (domains,  $\alpha$ -helices,  $\beta$ -sheets, framework regions of antibodies, etc.). A structural sub-element can be identified using known structures of similar or homologous (poly)peptides, or by using the above mentioned matrices of aligned amino acid sequences. Here the variability at each position is the basis for determining stretches of amino acid residues which belong to a structural sub-element (e.g. hypervariable regions of an antibody).

Sub-sequence:

A sub-sequence is defined as a genetic module which is flanked by unique cleavage sites and encodes at least one structural sub-element. It is not necessarily identical to a structural sub-element.

Cleavage site:

A short DNA sequence which is used as a specific target for a reagent which cleaves DNA in a sequence-specific manner (e.g. restriction endonucleases).

Compatible cleavage sites:

Cleavage sites are compatible with each other, if they can be efficiently ligated without modification and, preferably, also without adding an adapter molecule..

Unique cleavage sites:

A cleavage site is defined as unique if it occurs only once in a vector containing at least one of the genes of interest, or if a vector containing at least one of the genes of interest could be treated in a way that only one of the cleavage sites could be used by the cleaving agent.

Corresponding (poly)peptide sequences:

Sequences deduced from the same part of one group of homologous proteins are called corresponding (poly)peptide sequences.

Common cleavage sites:

A cleavage site in at least two corresponding sequences, which occurs at the same functional position (i.e. which flanks a defined sub-sequence), which can be hydrolyzed by the same cleavage tool and which yields identical compatible ends is termed a common cleavage site.

Excising genetic sub-sequences:

A method which uses the unique cleavage sites and the corresponding cleavage reagents to cleave the target DNA at the specified positions in order to isolate, remove or replace the genetic sub-sequence flanked by these unique cleavage sites.

Exchanging genetic sub-sequences:

A method by which an existing sub-sequence is removed using the flanking cleavage sites of this sub-sequence, and a new sub-sequence or a collection of sub-sequences, which contain ends compatible with the cleavage sites thus created, is inserted.

Expression of genes:

The term expression refers to in vivo or in vitro processes, by which the information of a gene is transcribed into mRNA and then translated into a protein/(poly)peptide. Thus, the term expression refers to a process which occurs inside cells, by which the information of a gene is transcribed into mRNA and then into a protein. The term expression also includes all events of post-translational modification and transport, which are necessary for the (poly)peptide to be functional.

Screening of protein/(poly)peptide libraries:

Any method which allows isolation of one or more proteins/(poly)peptides having a desired property from other proteins/(poly)peptides within a library.

Amino acid pattern characteristic for a species:

A (poly)peptide sequence is assumed to exhibit an amino acid pattern characteristic for a species if it is deduced from a collection of homologous proteins from just this species.

Immunoglobulin superfamily (IgSF):

The IgSF is a family of proteins comprising domains being characterized by the immunoglobulin fold. The IgSF comprises for example T-cell receptors and the immunoglobulins (antibodies).

Antibody framework:

A framework of an antibody variable domain is defined by Kabat et al. (1991) as the part of the variable domain which serves as a scaffold for the antigen binding loops of this variable domain.

Antibody CDR:

The CDRs (complementarity determining regions) of an antibody consist of the antigen binding loops, as defined by Kabat et al. (1991). Each of the two variable domains of an antibody Fv fragment contain three CDRs.

HuCAL:

Acronym for Human Combinatorial Antibody Library. Antibody Library based on modular consensus genes according to the invention (see Example 1).

Antibody fragment:

Any portion of an antibody which has a particular function, e.g. binding of antigen. Usually, antibody fragments are smaller than whole antibodies. Examples are Fv, disulphide-linked Fv, single-chain Fv (scFv), or Fab fragments. Additionally, antibody fragments are often engineered to include new functions or properties.

Universal framework:

One single framework which can be used to create the full variability of functions, specificities or properties which is originally sustained by a large collection of different frameworks, is called universal framework.

Binding of an antibody to its target:

The process which leads to a tight and specific association between an antibody and a corresponding molecule or ligand is called binding. A molecule or ligand or any part of a molecule or ligand which is recognized by an antibody is called the target.

Replacing genetic sub-sequences

A method by which an existing sub-sequence is removed using the flanking cleavage sites of this sub-sequence, and a new sub-sequence or collection of sub-

sequences, which contains ends compatible with the cleavage sites thus created, is inserted.

Assembling of genetic sequences:

Any process which is used to combine synthetic or natural genetic sequences in a specific manner in order to get longer genetic sequences which contain at least parts of the used synthetic or natural genetic sequences.

Analysis of homologous genes:

The corresponding amino acid sequences of two or more genes are aligned to each other in a way which maximizes the correspondence between identical or similar amino acid residues at all positions. These aligned sequences are termed homologous if the percentage of the sum of identical and/or similar residues exceeds a defined threshold. This threshold is commonly regarded by those skilled in the art as being exceeded when at least 15 per cent of the amino acids in the aligned genes are identical, and at least 30 per cent are similar.

***Legends to Figures and Tables***

- Fig. 1: Flow chart outlining the process of construction of a synthetic human antibody library based on consensus sequences.
- Fig. 2: Alignment of consensus sequences designed for each subgroup (amino acid residues are shown with their standard one-letter abbreviation). (A) kappa sequences, (B) lambda sequences and (C), heavy chain sequences. The positions are numbered according to Kabat (1991). In order to maximize homology in the alignment, gaps (—) have been introduced in the sequence at certain positions.
- Fig. 3: Gene sequences of the synthetic V kappa consensus genes. The corresponding amino acid sequences (see Fig. 2) as well as the unique cleavage sites are also shown.
- Fig. 4: Gene sequences of the synthetic V lambda consensus genes. The corresponding amino acid sequences (see Fig. 2) as well as the unique cleavage sites are also shown.
- Fig. 5: Gene sequences of the synthetic V heavy chain consensus genes. The corresponding amino acid sequences (see Fig. 2) as well as the unique cleavage sites are also shown.
- Fig. 6: Oligonucleotides used for construction of the consensus genes. The oligos are named according to the corresponding consensus gene, e.g. the gene V $\kappa$ 1 was constructed using the six oligonucleotides O1K1 to O1K6. The oligonucleotides used for synthesizing the genes encoding the constant domains C $\kappa$  (OCLK1 to 8) and CH1 (OCH1 to 8) are also shown.
- Fig. 7 A/B: Sequences of the synthetic genes encoding the constant domains C $\kappa$  (A) and CH1 (B). The corresponding amino acid sequences as well as unique cleavage sites introduced in these genes are also shown.
- Fig. 7C: Functional map and sequence of module M24 comprising the synthetic C $\lambda$  gene segment (huCL lambda).
- Fig. 7D: Oligonucleotides used for synthesis of module M24.
- Fig. 8: Sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-V $\kappa$ 2. The signal sequence (amino acids 1 to 21) was derived from the *E. coli* phoA gene (Skerra &



Plückthun, 1988). Between the *phoA* signal sequence and the VH3 domain, a short sequence stretch encoding 4 amino acid residues (amino acid 22 to 25) has been inserted in order to allow detection of the single-chain fragment in Western blot or ELISA using the monoclonal antibody M1 (Knappik & Plückthun, 1994). The last 6 basepairs of the sequence were introduced for cloning purposes (EcoRI site).

**Fig. 9:** Plasmid map of the vector pIG10.3 used for phage display of the H3k2 scFv fragment. The vector is derived from pIG10 and contains the gene for the lac operon repressor, *lacI*, the artificial operon encoding the H3k2-gene3ss fusion under control of the lac promoter, the *lpp* terminator of transcription, the single-strand replication origin of the *E. coli* phage f1 (F1\_ORI), a gene encoding  $\beta$ -lactamase (*bla*) and the ColEI derived origin of replication.

**Fig. 10:** Sequencing results of independent clones from the initial library, translated into the corresponding amino acid sequences. (A) Amino acid sequence of the VH3 consensus heavy chain CDR3 (position 93 to 102, Kabat numbering). (B) Amino acid sequences of 12 clones of the 10-mer library. (C) Amino acid sequences of 11 clones of the 15-mer library, \*: single base deletion.

**Fig. 11:** Expression test of individual library members. (A) Expression of 9 independent clones of the 10-mer library. (B) Expression of 9 independent clones of the 15-mer library. The lane designated with M contains the size marker. Both the gp3-scFv fusion and the scFv monomer are indicated.

**Fig. 12:** Enrichment of specific phage antibodies during the panning against FITC-BSA. The initial as well as the subsequent fluorescein-specific sub-libraries were panned against the blocking buffer and the ratio of the phage eluted from the FITC-BSA coated well vs. that from the powder milk coated well from each panning round is presented as the „specificity factor“.

**Fig. 13:** Phage ELISA of 24 independent clones after the third round of panning tested for binding on FITC-BSA.

**Fig. 14:** Competition ELISA of selected FITC-BSA binding clones. The ELISA signals ( $OD_{405nm}$ ) of scFv binding without inhibition are taken as 100%.

**Fig. 15:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against FITC-BSA, translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering).

- Fig. 16:** Coomassie-Blue stained SDS-PAGE of the purified anti-fluorescein scFv fragments: M: molecular weight marker, A: total soluble cell extract after induction, B: fraction of the flow-through, C, D and E: purified scFv fragments 1HA-3E4, 1HA-3E5 and 1HA-3E10, respectively.
- Fig. 17:** Enrichment of specific phage antibodies during the panning against  $\beta$ -estradiol-BSA, testosterone-BSA, BSA, ESL-1, interleukin-2, lymphotoxin- $\beta$ , and LeY-BSA after three rounds of panning.
- Fig. 18:** ELISA of selected ESL-1 and  $\beta$ -estradiol binding clones
- Fig. 19:** Selectivity and cross-reactivity of HuCAL antibodies: in the diagonal specific binding of HuCAL antibodies can be seen, off-diagonal signals show non-specific cross-reactivity.
- Fig. 20:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against  $\beta$ -estradiol-BSA, translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering). One clone is derived from the 10mer library.
- Fig. 21:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against testosterone-BSA, translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering).
- Fig. 22:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against lymphotoxin- $\beta$ , translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering). One clone comprises a 14mer CDR, presumably introduced by incomplete coupling of the trinucleotide mixture during oligonucleotide synthesis.
- Fig. 23:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against ESL-1, translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering). Two clones are derived from the 10mer library. One clone comprises a 16mer CDR, presumably introduced by chain elongation during oligonucleotide synthesis using trinucleotides.
- Fig. 24:** Sequencing results of the heavy chain CDR3s of independent clones after 3 rounds of panning against BSA, translated into the corresponding amino acid sequences (position 93 to 102, Kabat numbering).
- Fig. 25:** Schematic representation of the modular pCAL vector system.
- Fig. 25a:** List of restriction sites already used in or suitable for the modular HuCAL genes and pCAL vector system.
- Fig. 26:** List of the modular vector elements for the pCAL vector series: shown are only those restriction sites which are part of the modular system.

- Fig. 27: Functional map and sequence of the multi-cloning site module (MCS)
- Fig. 28: Functional map and sequence of the pMCS cloning vector series.
- Fig. 29: Functional map and sequence of the pCAL module M1 (see Fig. 26).
- Fig. 30: Functional map and sequence of the pCAL module M7-III (see Fig. 26).
- Fig. 31: Functional map and sequence of the pCAL module M9-II (see Fig. 26).
- Fig. 32: Functional map and sequence of the pCAL module M11-II (see Fig. 26).
- Fig. 33: Functional map and sequence of the pCAL module M14-Ext2 (see Fig. 26).
- Fig. 34: Functional map and sequence of the pCAL module M17 (see Fig. 26).
- Fig. 35: Functional map and sequence of the modular vector pCAL4.
- Fig. 35a: Functional maps and sequences of additional pCAL modules (M2, M3, M7I, M7II, M8, M10II, M11II, M12, M13, M19, M20, M21, M41) and of low-copy number plasmid vectors (pCALO1 to pCALO3).
- Fig. 35b: List of oligonucleotides and primers used for synthesis of pCAL vector modules.
- Fig. 36: Functional map and sequence of the  $\beta$ -lactamase cassette for replacement of CDRs for CDR library cloning.
- Fig. 37: Oligo and primer design for V $\kappa$  CDR3 libraries
- Fig. 38: Oligo and primer design for V $\lambda$  CDR3 libraries
- Fig. 39: Functional map of the pBS13 expression vector series.
- Fig. 40: Expression of all 49 HuCAL scFvs obtained by combining each of the 7 VH genes with each of the 7 VL genes (pBS13, 30°C): Values are given for the percentage of soluble vs. insoluble material, the total and the soluble amount compared to the combination H3 $\kappa$ 2, which was set to 100%. In addition, the corresponding values for the McPC603 scFv are given.

**Table 1:** Summary of human immunoglobulin germline sequences used for computing the germline membership of rearranged sequences. (A) kappa sequences, (B) lambda sequences and (C), heavy chain sequences. (1) The germline name used in the various calculations, (2) the references number for the corresponding sequence (see appendix for sequence related citations), (3) the family where each sequence belongs to and (4), the various names found in literature for germline genes with identical amino acid sequences.

**Table 2:** Rearranged human sequences used for the calculation of consensus sequences. (A) kappa sequences, (B) lambda sequences and (C), heavy chain sequences. The table summarized the name of the sequence (1),

the length of the sequence in amino acids (2), the germline family (3) as well as the computed germline counterpart (4). The number of amino acid exchanges between the rearranged sequence and the germline sequence is tabulated in (5), and the percentage of different amino acids is given in (6). Column (7) gives the references number for the corresponding sequence (see appendix for sequence related citations).

**Table 3:** Assignment of rearranged V sequences to their germline counterparts. (A) kappa sequences, (B) lambda sequences and (C), heavy chain sequences. The germline genes are tabulated according to their family (1), and the number of rearranged genes found for every germline gene is given in (2).

**Table 4:** Computation of the consensus sequence of the rearranged V kappa sequences. (A), V kappa subgroup 1, (B), V kappa subgroup 2, (C), V kappa subgroup 3 and (D), V kappa subgroup 4. The number of each amino acid found at each position is tabulated together with the statistical analysis of the data. (1) Amino acids are given with their standard one-letter abbreviations (and B means D or N, Z means E or Q and X means any amino acid). The statistical analysis summarizes the number of sequences found at each position (2), the number of occurrences of the most common amino acid (3), the amino acid residue which is most common at this position (4), the relative frequency of the occurrence of the most common amino acid (5) and the number of different amino acids found at each position (6).

**Table 5:** Computation of the consensus sequence of the rearranged V lambda sequences. (A), V lambda subgroup 1, (B), V lambda subgroup 2, and (C), V lambda subgroup 3. The number of each amino acid found at each position is tabulated together with the statistical analysis of the data. Abbreviations are the same as in Table 4.

**Table 6:** Computation of the consensus sequence of the rearranged V heavy chain sequences. (A), V heavy chain subgroup 1A, (B), V heavy chain subgroup 1B, (C), V heavy chain subgroup 2, (D), V heavy chain subgroup 3, (E), V heavy chain subgroup 4, (F), V heavy chain subgroup 5, and (G), V heavy chain subgroup 6. The number of each amino acid found at each position is tabulated together with the statistical analysis of the data. Abbreviations are the same as in Table 4.

## Examples

### Example 1: Design of a Synthetic Human Combinatorial Antibody Library (HuCAL)

The following example describes the design of a fully synthetic human combinatorial antibody library (HuCAL), based on consensus sequences of the human immunoglobulin repertoire, and the synthesis of the consensus genes. The general procedure is outlined in Fig. 1.

#### *1.1 Sequence database*

##### **1.1.1 Collection and alignment of human immunoglobulin sequences**

In a first step, sequences of variable domains of human immunoglobulins have been collected and divided into three sub bases: V heavy chain (VH), V kappa (Vκ) and V lambda (Vλ). For each sequence, the gene sequence was then translated into the corresponding amino acid sequence. Subsequently, all amino acid sequences were aligned according to Kabat et al. (1991). In the case of Vλ sequences, the numbering system of Chuchana et al. (1990) was used. Each of the three main databases was then divided into two further sub bases: the first sub base contained all sequences derived from rearranged V genes, where more than 70 positions of the sequence were known. The second sub base contained all germline gene segments (without the D- and J- minigenes; pseudogenes with internal stop codons were also removed). In all cases, where germline sequences with identical amino acid sequence but different names were found, only one sequence was used (see Table 1). The final databases of rearranged sequences contained 386, 149 and 674 entries for Vκ, Vλ and VH, respectively. The final databases of germline sequences contained 48, 26 and 141 entries for Vκ, Vλ and VH, respectively.

##### **1.1.2 Assignment of sequences to subgroups**

The sequences in the three germline databases were then grouped according to sequence homology (see also Tomlinson et al., 1992, Williams & Winter, 1993, and Cox et al., 1994). In the case of Vκ, 7 families could be established. Vλ was divided into 8 families and VH into 6 families. The VH germline genes of the VH7 family (Van Dijk et al., 1993) were grouped into the VH1 family, since the genes of the two families are highly homologous. Each family contained different numbers of germline genes, varying from 1 (for example VH6) to 47 (VH3).

## **1.2 Analysis of sequences**

### **1.2.1 Computation of germline membership**

For each of the 1209 amino acid sequences in the databases of rearranged genes, the nearest germline counterpart, i.e. the germline sequence with the smallest number of amino acid differences was then calculated. After the germline counterpart was found, the number of somatic mutations which occurred in the rearranged gene and which led to amino acid exchanges could be tabulated. In 140 cases, the germline counterpart could not be calculated exactly, because more than one germline gene was found with an identical number of amino acid exchanges. These rearranged sequences were removed from the database. In a few cases, the number of amino acid exchanges was found to be unusually large (>20 for VL and >25 for VH), indicating either heavily mutated rearranged genes or derivation from germline genes not present in the database. Since it was not possible to distinguish between these two possibilities, these sequences were also removed from the database. Finally, 12 rearranged sequences were removed from the database because they were found to have very unusual CDR lengths and composition or unusual amino acids at canonical positions (see below). In summary, 1023 rearranged sequences out of 1209 (85%) could be clearly assigned to their germline counterparts (see Table 2).

After this calculation, every rearranged gene could be arranged in one of the families established for the germline genes. Now the usage of each germline gene, i.e. the number of rearranged genes which originate from each germline gene, could be calculated (see Table 2). It was found that the usage was strongly biased towards a subset of germline genes, whereas most of the germline genes were not present as rearranged genes in the database and therefore apparently not used in the immune system (Table 3). This observation had already been reported in the case of V $\kappa$  (Cox, et al., 1994). All germline gene families, where no or only very few rearranged counterparts could be assigned, were removed from the database, leaving 4 V $\kappa$ , 3 V $\lambda$ , and 6 VH families.

### **1.2.2 Analysis of CDR conformations**

The conformation of the antigen binding loops of antibody molecules, the CDRs, is strongly dependent on both the length of the CDRs and the amino acid residues located at the so-called canonical positions (Chothia & Lesk, 1987). It has been found that only a few canonical structures exist, which determine the structural

repertoire of the immunoglobulin variable domains (Chothia et al., 1989). The canonical amino acid positions can be found in CDR as well as framework regions. The 13 used germline families defined above (7 VL and 6 VH) were now analyzed for their canonical structures in order to define the structural repertoire encoded in these families.

In 3 of the 4 V $\kappa$  families (V $\kappa$ 1, 2 and 4), one different type of CDR1 conformation could be defined for every family. The family V $\kappa$ 3 showed two types of CDR1 conformation: one type which was identical to V $\kappa$ 1 and one type only found in V $\kappa$ 3. All V $\kappa$  CDR2s used the same type of canonical structure. The CDR3 conformation is not encoded in the germline gene segments. Therefore, the 4 V $\kappa$  families defined by sequence homology and usage corresponded also to 4 types of canonical structures found in V $\kappa$  germline genes.

The 3 V $\lambda$  families defined above showed 3 types of CDR1 conformation, each family with one unique type. The V $\lambda$ 1 family contained 2 different CDR1 lengths (13 and 14 amino acids), but identical canonical residues, and it is thought that both lengths adopt the same canonical conformation (Chothia & Lesk, 1987). In the CDR2 of the used V $\lambda$  germlines, only one canonical conformation exists, and the CDR3 conformation is not encoded in the germline gene segments. Therefore, the 3 V $\lambda$  families defined by sequence homology and usage corresponded also to 3 types of canonical structures.

The structural repertoire of the human VH sequences was analyzed in detail by Chothia et al., 1992. In total, 3 conformations of CDR1 (H1-1, H1-2 and H1-3) and 6 conformations of CDR2 (H2-1, H2-2, H2-3, H2-4, H2-5 and H2-x) could be defined. Since the CDR3 is encoded in the D- and J-minigene segments, no particular canonical residues are defined for this CDR.

All the members of the VH1 family defined above contained the CDR1 conformation H1-1, but differed in their CDR2 conformation: the H2-2 conformation was found in 6 germline genes, whereas the conformation H2-3 was found in 8 germline genes. Since the two types of CDR2 conformations are defined by different types of amino acid at the framework position 72, the VH1 family was divided into two subfamilies: VH1A with CDR2 conformation H2-2 and VH1B with the conformation H2-3. The members of the VH2 family all had the conformations H1-3 and H2-1 in CDR1 and CDR2, respectively. The CDR1 conformation of the VH3 members was found in all cases to be H1-1, but 4 different types were found in CDR2 (H2-1, H2-3, H2-4 and H2-x). In these CDR2 conformations, the canonical framework residue 71 is always

defined by an arginine. Therefore, it was not necessary to divide the VH3 family into subfamilies, since the 4 types of CDR2 conformations were defined solely by the CDR2 itself. The same was true for the VH4 family. Here, all 3 types of CDR1 conformations were found, but since the CDR1 conformation was defined by the CDR itself (the canonical framework residue 26 was found to be glycine in all cases), no subdivisions were necessary. The CDR2 conformation of the VH4 members was found to be H2-1 in all cases. All members of the VH5 family were found to have the conformation H1-1 and H2-2, respectively. The single germline gene of the VH6 family had the conformations H1-3 and H2-5 in CDR1 and CDR2, respectively.

In summary, all possible CDR conformations of the V $\kappa$  and V $\lambda$  genes were present in the 7 families defined by sequence comparison. From the 12 different CDR conformations found in the used VH germline genes, 7 could be covered by dividing the family VH1 into two subfamilies, thereby creating 7 VH families. The remaining 5 CDR conformations (3 in the VH3 and 2 in the VH4 family) were defined by the CDRs themselves and could be created during the construction of CDR libraries. Therefore, the structural repertoire of the used human V genes could be covered by 49 (7 x 7) different frameworks.

### 1.2.3 Computation of consensus sequences

The 14 databases of rearranged sequences (4 V $\kappa$ , 3 V $\lambda$  and 7 VH) were used to compute the HuCAL consensus sequences of each subgroup (4 HuCAL- V $\kappa$ , 3 HuCAL- V $\lambda$ , 7 HuCAL- VH, see Table 4, 5 and 6). This was done by counting the number of amino acid residues used at each position (position variability) and subsequently identifying the amino acid residue most frequently used at each position. By using the rearranged sequences instead of the used germline sequences for the calculation of the consensus, the consensus was weighted according to the frequency of usage. Additionally, frequently mutated and highly conserved positions could be identified. The consensus sequences were cross-checked with the consensus of the germline families to see whether the rearranged sequences were biased at certain positions towards amino acid residues which do not occur in the collected germline sequences, but this was found not to be the case. Subsequently, the number of differences of each of the 14 consensus sequences to each of the germline sequences found in each specific family was calculated. The overall deviation from the most homologous germline sequence was found to be 2.4 amino acid residues (s.d. = 2.7), ensuring that the "artificial" consensus sequences



can still be considered as truly human sequences as far as immunogenicity is concerned.

### 1.3 Structural analysis

So far, only sequence information was used to design the consensus sequences. Since it was possible that during the calculation certain artificial combinations of amino acid residues have been created, which are located far away in the sequence but have contacts to each other in the three dimensional structure, leading to destabilized or even misfolded frameworks, the 14 consensus sequences were analyzed according to their structural properties.

It was rationalized that all rearranged sequences present in the database correspond to functional and therefore correctly folded antibody molecules. Hence, the most homologous rearranged sequence was calculated for each consensus sequence. The positions where the consensus differed from the rearranged sequence were identified as potential "artificial residues" and inspected.

The inspection itself was done in two directions. First, the local sequence stretch around each potentially "artificial residue" was compared with the corresponding stretch of all the rearranged sequences. If this stretch was found to be truly artificial, i.e. never occurred in any of the rearranged sequences, the critical residue was converted into the second most common amino acid found at this position and analyzed again. Second, the potentially "artificial residues" were analyzed for their long range interactions. This was done by collecting all available structures of human antibody variable domains from the corresponding PDB files and calculating for every structure the number and type of interactions each amino acid residue established to each side-chain. These "interaction maps" were used to analyze the probable side-chain/side-chain interactions of the potentially "artificial residues". As a result of this analysis, the following residues were exchanged (given is the name of the gene, the position according to Kabat's numbering scheme, the amino acid found at this position as the most abundant one and the amino acid which was used instead):

VH2: S<sub>65</sub>T

Vκ1: N<sub>34</sub>A,

Vκ3: G<sub>9</sub>A, D<sub>60</sub>A, R<sub>77</sub>S

Vλ3: V<sub>78</sub>T

#### 1.4 Design of CDR sequences

The process described above provided the complete consensus sequences derived solely from the databases of rearranged sequences. It was rationalized that the CDR1 and CDR2 regions should be taken from the databases of used germline sequences, since the CDRs of rearranged and mutated sequences are biased towards their particular antigens. Moreover, the germline CDR sequences are known to allow binding to a variety of antigens in the primary immune response, where only CDR3 is varied. Therefore, the consensus CDRs obtained from the calculations described above were replaced by germline CDRs in the case of VH and V $\kappa$ . In the case of V $\lambda$ , a few amino acid exchanges were introduced in some of the chosen germline CDRs in order to avoid possible protease cleavage sites as well as possible structural constraints.

The CDRs of following germline genes have been chosen:

<u>HuCAL gene</u>	<u>CDR1</u>	<u>CDR2</u>
HuCAL-VH1A	VH1-12-1	VH1-12-1
HuCAL-VH1B	VH1-13-16	VH1-13-6,-7,-8,-9
HuCAL-VH2	VH2-31-10,-11,-12,-13	VH2-31-3,-4
HuCAL-VH3	VH3-13-8,-9,-10	VH3-13-8,-9,-10
HuCAL-VH4	VH4-11-7 to -14	VH4-11-8,-9,-11,-12,-14,-16 VH4-31-17,-18,-19,-20
HuCAL-VH5	VH5-12-1,-2	VH5-12-1,-2
HuCAL-VH6	VH6-35-1	VH6-35-1
HuCAL-V $\kappa$ 1	V $\kappa$ 1-14,-15	V $\kappa$ 1-2,-3,-4,-5,-7,-8,-12,-13,-18,-19
HuCAL-V $\kappa$ 2	V $\kappa$ 2-6	V $\kappa$ 2-6
HuCAL-V $\kappa$ 3	V $\kappa$ 3-1,-4	V $\kappa$ 3-4
HuCAL-V $\kappa$ 4	V $\kappa$ 4-1	V $\kappa$ 4-1
HuCAL-V $\lambda$ 1	HUMLV117,DPL5	DPL5
HuCAL-V $\lambda$ 2	DPL11,DPL12	DPL12
HuCAL-V $\lambda$ 3	DPL23	HUMLV318

In the case of the CDR3s, any sequence could be chosen since these CDRs were planned to be the first to be replaced by oligonucleotide libraries. In order to study the expression and folding behavior of the consensus sequences in *E. coli*, it would be useful to have all sequences with the same CDR3, since the influence of the CDR3s on the folding behavior would then be identical in all cases. The dummy sequences QQHYTTPP and ARWGGDGFYAMDY were selected for the VL chains (kappa and lambda) and for the VH chains, respectively. These sequences are known to be compatible with antibody folding in *E. coli* (Carter et al., 1992).

### 1.5 Gene design

The final outcome of the process described above was a collection of 14 HuCAL amino acid sequences, which represent the frequently used structural antibody repertoire of the human immune system (see Figure 2). These sequences were back-translated into DNA sequences. In a first step, the back-translation was done using only codons which are known to be frequently used in *E. coli*. These gene sequences were then used for creating a database of all possible restriction endonuclease sites, which could be introduced without changing the corresponding amino acid sequences. Using this database, cleavage sites were selected which were located at the flanking regions of all sub-elements of the genes (CDRs and framework regions) and which could be introduced in all HuCAL VH, V $\kappa$  or V $\lambda$  genes simultaneously at the same position. In a few cases it was not possible to find cleavage sites for all genes of a subgroup. When this happened, the amino acid sequence was changed, if this was possible according to the available sequence and structural information. This exchange was then analyzed again as described above. In total, the following 6 amino acid residues were exchanged during this design (given is the name of the gene, the position according to Kabat's numbering scheme, the amino acid found at this position as the most abundant one and the amino acid which was used instead):

VH2: T<sub>3</sub>Q

VH6: S<sub>42</sub>G

V $\kappa$ 3: E<sub>1</sub>D, I<sub>58</sub>V

V $\kappa$ 4: K<sub>24</sub>R

V $\lambda$ 3: T<sub>22</sub>S

In one case (5'-end of VH framework 3) it was not possible to identify a single cleavage site for all 7 VH genes. Two different type of cleavage sites were used instead: BstEII for HuCAL VH1A, VH1B, VH4 and VH5, and NspV for HuCAL VH2, VH3, VH4 and VH6.

Several restriction endonuclease sites were identified, which were not located at the flanking regions of the sub-elements but which could be introduced in every gene of a given group without changing the amino acid sequence. These cleavage sites were also introduced in order to make the system more flexible for further improvements. Finally, all but one remaining restriction endonuclease sites were removed in every gene sequence. The single cleavage site, which was not removed was different in all genes of a subgroup and could be therefore used as a "fingerprint" site to ease the identification of the different genes by restriction digest. The designed genes, together with the corresponding amino acid sequences and the group-specific restriction endonuclease sites are shown in Figure 3, 4 and 5, respectively.

### ***1.6 Gene synthesis and cloning***

The consensus genes were synthesized using the method described by Prodromou & Pearl, 1992, using the oligonucleotides shown in Fig. 6. Gene segments encoding the human constant domains C $\kappa$ , C $\lambda$  and CH1 were also synthesized, based on sequence information given by Kabat et al., 1991 (see Fig. 6 and Fig. 7). Since for both the CDR3 and the framework 4 gene segments identical sequences were chosen in all HuCAL V $\kappa$ , V $\lambda$  and VH genes, respectively, this part was constructed only once, together with the corresponding gene segments encoding the constant domains. The PCR products were cloned into pCR-Script KS(+) (Stratagene, Inc.) or pZER0-1 (Invitrogen, Inc.) and verified by sequencing.

### **Example 2: Cloning and Testing of a HuCAL-Based Antibody Library**

A combination of two of the synthetic consensus genes was chosen after construction to test whether binding antibody fragments can be isolated from a library based on these two consensus frameworks. The two genes were cloned as a single-chain Fv (scFv) fragment, and a VH-CDR3 library was inserted. In order to test the library for the presence of functional antibody molecules, a selection procedure

was carried out using the small hapten fluorescein bound to BSA (FITC-BSA) as antigen.

### ***2.1 Cloning of the HuCAL VH3-Vk2 scFv fragment***

In order to test the design of the consensus genes, one randomly chosen combination of synthetic light and heavy gene (HuCAL-Vk2 and HuCAL-VH3) was used for the construction of a single-chain antibody (scFv) fragment. Briefly, the gene segments encoding the VH3 consensus gene and the CH1 gene segment including the CDR3 - framework 4 region, as well as the Vk2 consensus gene and the C $\kappa$  gene segment including the CDR3 - framework 4 region were assembled yielding the gene for the VH3-CH1 Fd fragment and the gene encoding the Vk2-C $\kappa$  light chain, respectively. The CH1 gene segment was then replaced by an oligonucleotide cassette encoding a 20-mer peptide linker with the sequence AGGGSGGGSGGGSGGGGS. The two oligonucleotides encoding this linker were 5'-TCAGCGGGTGGCGGTTCTGGCGGCGGTGGGAGCGGTGGCGGTGGTTC-TGGCGGTGGTGGTTCGATATCGGTCCACGTACGG-3' and 5'-AATTCCGTACG-TGGACCGATATCGGAACCACCACCGCCAGAACCACCGCCACCGCTCCACCGCCGCGCCAGAACCGCCACCCGC-3', respectively. Finally, the HuCAL-Vk2 gene was inserted via EcoRV and BsiWI into the plasmid encoding the HuCAL-VH3-linker fusion, leading to the final gene HuCAL-VH3-Vk2, which encoded the two consensus sequences in the single-chain format VH-linker-VL. The complete coding sequence is shown in Fig. 8.

### ***2.2 Construction of a monovalent phage-display phagemid vector pIG10.3***

Phagemid pIG10.3 (Fig. 9) was constructed in order to create a phage-display system (Winter et al., 1994) for the H3 $\kappa$ 2 scFv gene. Briefly, the EcoRI/HindIII restriction fragment in the phagemid vector pIG10 (Ge et al., 1995) was replaced by the c-myc followed by an amber codon (which encodes an glutamate in the amber-suppressor strain XL1 Blue and a stop codon in the non-suppressor strain JM83) and a truncated version of the gene III (fusion junction at codon 249, see Lowman et al., 1991) through PCR mutagenesis.

### 2.3 Construction of H-CDR3 libraries

Heavy chain CDR3 libraries of two lengths (10 and 15 amino acids) were constructed using trinucleotide codon containing oligonucleotides (Virnekås et al., 1994) as templates and the oligonucleotides complementing the flanking regions as primers. To concentrate only on the CDR3 structures that appear most often in functional antibodies, we kept the salt-bridge of R<sub>H94</sub> and D<sub>H101</sub> in the CDR3 loop. For the 15-mer library, both phenylalanine and methionine were introduced at position 100 since these two residues were found to occur quite often in human CDR3s of this length (not shown). For the same reason, valine and tyrosine were introduced at position 102. All other randomized positions contained codons for all amino acids except cysteine, which was not used in the trinucleotide mixture.

The CDR3 libraries of lengths 10 and 15 were generated from the PCR fragments using oligonucleotide templates O3HCDR103T (5'- GATACGGCCGTGTATTA-TTGCGCGCGT (TRI)<sub>6</sub>GATTATTGGGGCCAAGGCACCCTG-3') and O3HCDR153T (5'-GATACGGCCGT GTATTATTGCGCGCGT(TRI)<sub>10</sub>(TTT/ATG)GAT(GTT/TAT)TGGG-GCCAAGGCACCCTG-3'), and primers O3HCDR35 (5'-GATACGGCCGTGTATTA-TTGC-3') and O3HCDR33 (5'-CAGGGTGCCTTGGCCCC-3'), where TRI are trinucleotide mixtures representing all amino acids without cysteine, (TTT/ATG) and (GTT/TAT) are trinucleotide mixtures encoding the amino acids phenylalanine/methionine and valine/tyrosine, respectively. The potential diversity of these libraries was  $4.7 \times 10^7$  and  $3.4 \times 10^{10}$  for 10-mer and 15-mer library, respectively. The library cassettes were first synthesized from PCR amplification of the oligo templates in the presence of both primers: 25 pmol of the oligo template O3HCDR103T or O3HCDR153T, 50 pmol each of the primers O3HCDR35 and O3HCDR33, 20 nmol of dNTP, 10x buffer and 2.5 units of Pfu DNA polymerase (Stratagene) in a total volume of 100 µl for 30 cycles (1 minute at 92°C, 1 minute at 62°C and 1 minute at 72°C). A hot-start procedure was used. The resulting mixtures were phenol-extracted, ethanol-precipitated and digested overnight with EagI and Styl. The vector pIG10.3-sch3k2cat, where the EagI-Styl fragment in the vector pIG10.3-sch3k2 encoding the H-CDR3 was replaced by the chloramphenicol acetyltransferase gene (cat) flanked with these two sites, was similarly digested. The digested vector (35 µg) was gel-purified and ligated with 100 µg of the library cassette overnight at 16°C. The ligation mixtures were isopropanol precipitated, air-dried and the pellets were redissolved in 100 µl of ddH<sub>2</sub>O. The ligation was mixed with 1 ml of freshly prepared electrocompetent XL1 Blue on ice. 20 rounds of electroporation were performed and the transformants were diluted in SOC medium, shaken at 37°C for 30 minutes and plated out on large LB plates (Amp/Tet/Glucose)

at 37°C for 6-9 hrs. The number of transformants (library size) was  $3.2 \times 10^7$  and  $2.3 \times 10^7$  for the 10-mer and the 15-mer library, respectively. The colonies were suspended in 2xYT medium (Amp/Tet/Glucose) and stored as glycerol culture.

In order to test the quality of the initial library, phagemids from 24 independent colonies (12 from the 10-mer and 12 from the 15-mer library, respectively) were isolated and analyzed by restriction digestion and sequencing. The restriction analysis of the 24 phagemids indicated the presence of intact vector in all cases. Sequence analysis of these clones (see Fig. 10) indicated that 22 out of 24 contained a functional sequence in their heavy chain CDR3 regions. 1 out of 12 clones of the 10-mer library had a CDR3 of length 9 instead of 10, and 2 out of 12 clones of the 15-mer library had no open reading frame, thereby leading to a non-functional scFv; one of these two clones contained two consecutive inserts, but out of frame (data not shown). All codons introduced were presented in an even distribution.

Expression levels of individual library members were also measured. Briefly, 9 clones from each library were grown in 2xYT medium containing Amp/Tet/0.5% glucose at 37°C overnight. Next day, the cultures were diluted into fresh medium with Amp/Tet. At an  $OD_{600nm}$  of 0.4, the cultures were induced with 1 mM of IPTG and shaken at RT overnight. Then the cell pellets were suspended in 1 ml of PBS buffer + 1 mM of EDTA. The suspensions were sonicated and the supernatants were separated on an SDS-PAGE under reducing conditions, blotted on nylon membrane and detected with anti-FLAG M1 antibody (see Fig. 11). From the nine clones of the 10-mer library, all express the scFv fragments. Moreover, the gene III / scFv fusion proteins were present in all cases. Among the nine clones from the 15-mer library analyzed, 6/9 (67%) led to the expression of both scFv and the gene III/scFv fusion proteins. More importantly, all clones expressing the scFvs and gene III/scFv fusions gave rise to about the same level of expression.

## **2.4 Biopanning**

Phages displaying the antibody libraries were prepared using standard protocols. Phages derived from the 10-mer library were mixed with phages from the 15-mer library in a ratio of 20:1 ( $1 \times 10^{10}$  cfu/well of the 10-mer and  $5 \times 10^8$  cfu/well of the 15-mer phages, respectively). Subsequently, the phage solution was used for panning in ELISA plates (Maxisorp, Nunc) coated with FITC-BSA (Sigma) at concentration of 100 µg/ml in PBS at 4°C overnight. The antigen-coated wells were blocked with 3% powder milk in PBS and the phage solutions in 1% powder milk were added to each

well and the plate was shaken at RT for 1 hr. The wells were then washed with PBST and PBS (4 times each with shaking at RT for 5 minutes). The bound phages were eluted with 0.1 M triethylamine (TEA) at RT for 10 minutes. The eluted phage solutions were immediately neutralized with 1/2 the volume of 1 M Tris-Cl, pH 7.6. Eluted phage solutions (ca. 450  $\mu$ l) were used to infect 5 ml of XL1 Blue cells at 37°C for 30 min. The infected cultures were then plated out on large LB plates (Amp/Tet/Glucose) and allowed to grow at 37°C until the colonies were visible. The colonies were suspended in 2xYT medium and the glycerol cultures were made as above described. This panning round was repeated twice, and in the third round elution was carried out with addition of fluorescein in a concentration of 100  $\mu$ g/ml in PBS. The enrichment of specific phage antibodies was monitored by panning the initial as well as the subsequent fluorescein-specific sub-libraries against the blocking buffer (Fig. 12). Antibodies with specificity against fluorescein were isolated after 3 rounds of panning.

## 2.5 ELISA measurements

One of the criteria for the successful biopanning is the isolation of individual phage clones that bind to the targeted antigen or hapten. We undertook the isolation of anti-FITC phage antibody clones and characterized them first in a phage ELISA format. After the 3rd round of biopanning (see above), 24 phagemid containing clones were used to inoculate 100  $\mu$ l of 2xYT medium (Amp/Tet/Glucose) in an ELISA plate (Nunc), which was subsequently shaken at 37°C for 5 hrs. 100  $\mu$ l of 2xYT medium (Amp/Tet/1 mM IPTG) were added and shaking was continued for 30 minutes. A further 100  $\mu$ l of 2xYT medium (Amp/Tet) containing the helper phage ( $1 \times 10^9$  cfu/well) was added and shaking was done at RT for 3 hrs. After addition of kanamycin to select for successful helper phage infection, the shaking was continued overnight. The plates were then centrifuged and the supernatants were pipetted directly into ELISA wells coated with 100  $\mu$ l FITC-BSA (100 $\mu$ g/ml) and blocked with milk powder. Washing was performed similarly as during the panning procedure and the bound phages were detected with anti-M13 antibody-POD conjugate (Pharmacia) using soluble POD substrate (Boehringer-Mannheim). Of the 24 clones screened against FITC-BSA, 22 were active in the ELISA (Fig. 13). The initial libraries of similar titer gave rise to no detectable signal. Specificity for fluorescein was measured in a competitive ELISA. Periplasmic fractions of five FITC specific scFvs were prepared as described above. Western blotting indicated that all clones expressed about the same amount of scFv fragment



(data not shown). ELISA was performed as described above, but additionally, the periplasmic fractions were incubated 30 min at RT either with buffer (no inhibition), with 10 mg/ml BSA (inhibition with BSA) or with 10 mg/ml fluorescein (inhibition with fluorescein) before adding to the well. Binding scFv fragment was detected using the anti-FLAG antibody M1. The ELISA signal could only be inhibited, when soluble fluorescein was added, indicating binding of the scFvs was specific for fluorescein (Fig. 14).

## 2.6 Sequence analysis

The heavy chain CDR3 region of 20 clones were sequenced in order to estimate the sequence diversity of fluorescein binding antibodies in the library (Fig. 15). In total, 16 of 20 sequences (80%) were different, showing that the constructed library contained a highly diverse repertoire of fluorescein binders. The CDR3s showed no particular sequence homology, but contained on average 4 arginine residues. This bias towards arginine in fluorescein binding antibodies had already been described by Barbas et al., 1992.

## 2.7 Production

*E. coli* JM83 was transformed with phagemid DNA of 3 selected clones and cultured in 0.5 L 2xYT medium. Induction was carried out with 1 mM IPTG at  $OD_{600nm} = 0.4$  and growth was continued with vigorous shaking at RT overnight. The cells were harvested and pellets were suspended in PBS buffer and sonicated. The supernatants were separated from the cell debris via centrifugation and purified via the BioLogic system (Bio-Rad) by with a POROS<sup>®</sup>MC 20 column (IMAC, PerSeptive Biosystems, Inc.) coupled with an ion-exchange chromatography column. The ion-exchange column was one of the POROS<sup>®</sup>HS, CM or HQ or PI 20 (PerSeptive Biosystems, Inc.) depended on the theoretical pI of the scFv being purified. The pH of all the buffers was adjusted to one unit lower or higher than the pI of the scFv being purified throughout. The sample was loaded onto the first IMAC column, washed with 7 column volumes of 20 mM sodium phosphate, 1 M NaCl and 10 mM imidazole. This washing was followed by 7 column volumes of 20 mM sodium phosphate and 10 mM imidazole. Then 3 column volumes of an imidazole gradient (10 to 250 mM) were applied and the eluent was connected directly to the ion-exchanger. Nine column volumes of isocratic washing with 250 mM imidazole was followed by 15 column volumes of 250 mM to 100 mM and 7 column volumes of an imidazole / NaCl gradient (100 to 10 mM imidazole, 0 to 1 M NaCl). The flow rate was 5 ml/min. The purity of scFv fragments was checked by SDS-PAGE Coomassie

staining (Fig. 16). The concentration of the fragments was determined from the absorbance at 280 nm using the theoretically determined extinction coefficient (Gill & von Hippel, 1989). The scFv fragments could be purified to homogeneity (see Fig. 16). The yield of purified fragments ranged from 5 to 10 mg/L/OD.

### **Example 3: HuCAL H3κ2 Library Against a Collection of Antigens**

In order to test the library used in Example 2 further, a new selection procedure was carried out using a variety of antigens comprising  $\beta$ -estradiol, testosterone, Lewis-Y epitope (LeY), interleukin-2 (IL-2), lymphotoxin- $\beta$  (LT- $\beta$ ), E-selectin ligand-1 (ESL-1), and BSA.

#### **3.1 Biopanning**

The library and all procedures were identical to those described in Example 2. The ELISA plates were coated with  $\beta$ -estradiol-BSA (100  $\mu$ g/ml), testosterone-BSA (100  $\mu$ g/ml), LeY-BSA (20  $\mu$ g/ml) IL-2 (20  $\mu$ g/ml), ESL-1 (20  $\mu$ g/ml) and BSA (100  $\mu$ g/ml), LT- $\beta$  (denatured protein, 20  $\mu$ g/ml). In the first two rounds, bound phages were eluted with 0.1 M triethylamine (TEA) at RT for 10 minutes. In the case of BSA, elution after three rounds of panning was carried out with addition of BSA in a concentration of 100  $\mu$ g/ml in PBS. In the case of the other antigens, third round elution was done with 0.1 M triethylamine. In all cases except LeY, enrichment of binding phages could be seen (Figure 17). Moreover, a repetition of the biopanning experiment using only the 15-mer library resulted in the enrichment of LeY-binding phages as well (data not shown).

#### **3.2. ELISA measurements**

Clones binding to  $\beta$ -estradiol, testosterone, LeY, LT- $\beta$ , ESL-1 and BSA were further analyzed and characterized as described in Example 2 for FITC. ELISA data for anti- $\beta$ -estradiol and anti-ESL-1 antibodies are shown in Fig. 18. In one experiment, selectivity and cross-reactivity of binding scFv fragments were tested. For this purpose, an ELISA plate was coated with FITC, testosterone,  $\beta$ -estradiol, BSA, and ESL-1, with 5 wells for each antigen arranged in 5 rows, and 5 antibodies, one against each of the antigens, were screened against each of the antigens. Fig. 19

shows the specific binding of the antibodies to the antigen it was selected for, and the low cross-reactivity with the other four antigens.

### **3.3 Sequence analysis**

The sequencing data of several clones against  $\beta$ -estradiol (34 clones), testosterone (12 clones), LT- $\beta$  (23 clones), ESL-1 (34 clones), and BSA (10 clones) are given in Figures 20 to 24.

#### **Example 4: Vector Construction**

To be able to take advantage of the modularity of the consensus gene repertoire, a vector system had to be constructed which could be used in phage display screening of HuCAL libraries and subsequent optimization procedures. Therefore, all necessary vector elements such as origins of single-stranded or double-stranded replication, promotor/operator, repressor or terminator elements, resistance genes, potential recombination sites, gene III for display on filamentous phages, signal sequences, or detection tags had to be made compatible with the restriction site pattern of the modular consensus genes. Figure 25 shows a schematic representation of the pCAL vector system and the arrangement of vector modules and restriction sites therein. Figure 25a shows a list of all restriction sites which are already incorporated into the consensus genes or the vector elements as part of the modular system or which are not yet present in the whole system. The latter could be used in a later stage for the introduction of or within new modules.

#### **4.1 Vector modules**

A series of vector modules was constructed where the restriction sites flanking the gene sub-elements of the HuCAL genes were removed, the vector modules themselves being flanked by unique restriction sites. These modules were constructed either by gene synthesis or by mutagenesis of templates. Mutagenesis was done by add-on PCR, by site-directed mutagenesis (Kunkel et al., 1991) or multisite oligonucleotide-mediated mutagenesis (Sutherland et al., 1995; Perlak, 1990) using a PCR-based assembly method.

Figure 26 contains a list of the modules constructed. Instead of the terminator module M9 (HindIII-Ipp-PacI), a larger cassette M9II was prepared to introduce FseI as additional restriction site. M9II can be cloned via HindIII/BsrGI.

All vector modules were characterized by restriction analysis and sequencing. In the case of module M11-II, sequencing of the module revealed a two-base difference in positions 164/65 compared to the sequence database of the template. These two different bases (CA → GC) created an additional BanII site. Since the same two-base difference occurs in the f1 origin of other bacteriophages, it can be assumed that the two-base difference was present in the template and not created by mutagenesis during cloning. This BanII site was removed by site-directed mutagenesis, leading to module M11-III. The BssSI site of module M14 could initially not be removed without impact on the function of the ColE1 origin, therefore M14-Ext2 was used for cloning of the first pCAL vector series. Figures 29 to 34 are showing the functional maps and sequences of the modules used for assembly of the modular vector pCAL4 (see below). The functional maps and sequences of additional modules can be found in Figure 35a. Figure 35b contains a list of oligonucleotides and primers used for the synthesis of the modules.

#### **4.2 Cloning vector pMCS**

To be able to assemble the individual vector modules, a cloning vector pMCS containing a specific multi-cloning site (MCS) was constructed. First, an MCS cassette (Fig. 27) was made by gene synthesis. This cassette contains all those restriction sites in the order necessary for the sequential introduction of all vector modules and can be cloned via the 5'-HindIII site and a four base overhang at the 3'-end compatible with an AatII site. The vector pMCS (Figure 28) was constructed by digesting pUC19 with AatII and HindIII, isolating the 2174 base pair fragment containing the bla gene and the ColE1 origin, and ligating the MCS cassette.

#### **4.3 Cloning of modular vector pCAL4**

This was cloned step by step by restriction digest of pMCS and subsequent ligation of the modules M1 (via AatII/XbaI), M7III (via EcoRI/HindIII), and M9II (via HindIII/BsrGI), and M11-II (via BsrGI/NheI). Finally, the bla gene was replaced by the cat gene module M17 (via AatII/BglII), and the wild type ColE1 origin by module M14-Ext2 (via BglII/NheI). Figure 35 is showing the functional map and the sequence of pCAL4.

#### **4.4 Cloning of low-copy number plasmid vectors pCALO**

A series of low-copy number plasmid vectors was constructed in a similar way using the p15A module M12 instead of the ColE1 module M14-Ext2. Figure 35a is showing the functional maps and sequences of the vectors pCALO1 to pCALO3.

### **Example 5: Construction of a HuCAL scFv Library**

#### **5.1. Cloning of all 49 HuCAL scFv fragments**

All 49 combinations of the 7 HuCAL-VH and 7 HuCAL-VL consensus genes were assembled as described for the HuCAL VH3-V $\kappa$ 2 scFv in Example 2 and inserted into the vector pBS12, a modified version of the pLisc series of antibody expression vectors (Skerra et al., 1991).

#### **5.2 Construction of a CDR cloning cassette**

For replacement of CDRs, a universal  $\beta$ -lactamase cloning cassette was constructed having a multi-cloning site at the 5'-end as well as at the 3'-end. The 5'-multi-cloning site comprises all restriction sites adjacent to the 5'-end of the HuCAL VH and VL CDRs, the 3'-multi-cloning site comprises all restriction sites adjacent to the 3' end of the HuCAL VH and VL CDRs. Both 5'- and 3'-multi-cloning site were prepared as cassettes via add-on PCR using synthetic oligonucleotides as 5'- and 3'-primers using wild type  $\beta$ -lactamase gene as template. Figure 36 shows the functional map and the sequence of the cassette bla-MCS.

#### **5.3. Preparation of VL-CDR3 library cassettes**

The VL-CDR3 libraries comprising 7 random positions were generated from the PCR fragments using oligonucleotide templates V $\kappa$ 1&V $\kappa$ 3, V $\kappa$ 2 and V $\kappa$ 4 and primers O\_K3L\_5 and O\_K3L\_3 (Fig. 37) for the V $\kappa$  genes, and V $\lambda$  and primers O\_L3L\_5 (5'-GCAGAAGGCGAACGTCC-3') and O\_L3LA\_3 (Fig. 38) for the V $\lambda$  genes. Construction of the cassettes was performed as described in Example 2.3.

#### **5.4 Cloning of HuCAL scFv genes with VL-CDR3 libraries**

Each of the 49 single-chains was subcloned into pCAL4 via XbaI/EcoRI and the VL-CDR3 replaced by the  $\beta$ -lactamase cloning cassette via BbsI/MscI, which was then replaced by the corresponding VL-CDR3 library cassette synthesized as described above. This CDR replacement is described in detail in Example 2.3 where the cat gene was used.

#### **5.5 Preparation of VH-CDR3 library cassette**

The VH-CDR3 libraries were designed and synthesized as described in Example 2.3.

#### **5.6 Cloning of HuCAL scFv genes with VL- and VH-CDR3 libraries**

Each of the 49 single-chain VL-CDR3 libraries was digested with BssHII/StyI to replace VH-CDR3. The "dummy" cassette digested with BssHII/StyI was inserted, and was then replaced by a corresponding VH-CDR3 library cassette synthesized as described above.

#### **Example 6: Expression tests**

Expression and toxicity studies were performed using the scFv format VH-linker-VL. All 49 combinations of the 7 HuCAL-VH and 7 HuCAL-VL consensus genes assembled as described in Example 5 were inserted into the vector pBS13, a modified version of the pLisc series of antibody expression vectors (Skerra et al., 1991). A map of this vector is shown in Fig. 39.

*E. coli* JM83 was transformed 49 times with each of the vectors and stored as glycerol stock. Between 4 and 6 clones were tested simultaneously, always including the clone H3k2, which was used as internal control throughout. As additional control, the McPC603 scFv fragment (Knappik & Plückthun, 1995) in pBS13 was expressed under identical conditions. Two days before the expression test was performed, the clones were cultivated on LB plates containing 30  $\mu$ g/ml chloramphenicol and 60 mM glucose. Using this plates an 3 ml culture (LB medium

containing 90 µg chloramphenicol and 60 mM glucose) was inoculated overnight at 37 °C. Next day the overnight culture was used to inoculate 30 ml LB medium containing chloramphenicol (30 µg/ml). The starting OD<sub>600nm</sub> was adjusted to 0.2 and a growth temperature of 30 °C was used. The physiology of the cells was monitored by measuring every 30 minutes for 8 to 9 hours the optical density at 600 nm. After the culture reached an OD<sub>600nm</sub> of 0.5, antibody expression was induced by adding IPTG to a final concentration of 1 mM. A 5 ml aliquot of the culture was removed after 2 h of induction in order to analyze the antibody expression. The cells were lysed and the soluble and insoluble fractions of the crude extract were separated as described in Knappik & Plückthun, 1995. The fractions were assayed by reducing SDS-PAGE with the samples normalized to identical optical densities. After blotting and immunostaining using the α-FLAG antibody M1 as the first antibody (see Ge et al., 1994) and an Fc-specific anti-mouse antiserum conjugated to alkaline phosphatase as the second antibody, the lanes were scanned and the intensities of the bands of the expected size (appr. 30 kDa) were quantified densitometrically and tabulated relative to the control antibody (see Fig. 40).

## Example 7: Optimization of Fluorescein Binders

### 7.1. Construction of L-CDR3 and H-CDR2 library cassettes

A L-CDR3 library cassette was prepared from the oligonucleotide template CDR3L (5'-TGGAAGCTGAAGACGTGGGCGTGTATTATTGCCAGCAG(TR5)(TRI)<sub>4</sub>CCG(TRI)-TTTGGCCAGGGTACGAAAGTT-3') and primer 5'-AACTTTCGTACCCTGGCC-3' for synthesis of the complementary strand, where (TRI) was a trinucleotide mixture representing all amino acids except Cys, (TR5) comprised a trinucleotide mixture representing the 5 codons for Ala, Arg, His, Ser, and Tyr.

A H-CDR2 library cassette was prepared from the oligonucleotide template CDRsH (5'-AGGGTCTCGAGTGGGTGAGC(TRI)ATT(TRI)<sub>2,3</sub>(6)<sub>2</sub>(TRI)ACC(TRI)TATGCGGATAGCGTGAAAGGCCGTTTTACCATTTACGTGATAATTCGAAAAACACCA-3'), and primer 5'-TGGTGTTTTTCGAATTATCA-3' for synthesis of the complementary strand, where (TRI) was a trinucleotide mixture representing all amino acids except Cys, (6) comprised the incorporation of (A/G) (A/C/G) T, resulting in the formation of 6 codons for Ala, Asn, Asp, Gly, Ser, and Thr, and the length distribution being obtained by performing one substoichiometric coupling of the (TRI) mixture during synthesis, omitting the capping step normally used in DNA synthesis.

DNA synthesis was performed on a 40 nmole scale, oligos were dissolved in 1E buffer, purified via gel filtration using spin columns (S-200), and the DNA concentration determined by OD measurement at 260 nm (OD 1.0 = 40  $\mu$ g/ml).

10 nmole of the oligonucleotide templates and 12 nmole of the corresponding primers were mixed and annealed at 80°C for 1 min, and slowly cooled down to 37°C within 20 to 30 min. The fill-in reaction was performed for 2 h at 37°C using Klenow polymerase (2.0  $\mu$ l) and 250 nmole of each dNTP. The excess of dNTPs was removed by gel filtration using Nick-Spin columns (Pharmacia), and the double-stranded DNA digested with BbsI/MscI (L-CDR3), or XhoI/SfuI (H-CDR2) over night at 37°C. The cassettes were purified via Nick-Spin columns (Pharmacia), the concentration determined by OD measurement, and the cassettes aliquoted (15 pmole) for being stored at -80°C.

### **7.2 Library cloning:**

DNA was prepared from the collection of FITC binding clones obtained in Example 2 (approx.  $10^4$  to clones). The collection of scFv fragments was isolated via XbaI/EcoRI digest. The vector pCAL4 (100 fmole, 10  $\mu$ g) described in Example 4.3 was similarly digested with XbaI/EcoRI, gel-purified and ligated with 300 fmole of the scFv fragment collection over night at 16°C. The ligation mixture was isopropanol precipitated, air-dried, and the pellets were redissolved in 100  $\mu$ l of dd H<sub>2</sub>O. The ligation mixture was mixed with 1 ml of freshly prepared electrocompetent SCS 101 cells (for optimization of L-CDR3), or XL1 Blue cells (for optimization of H-CDR2) on ice. One round of electroporation was performed and the transformants were eluted in SOC medium, shaken at 37°C for 30 minutes, and an aliquot plated out on LB plates (Amp/Tet/Glucose) at 37°C for 6-9 hrs. The number of transformants was  $5 \times 10^4$ .

Vector DNA (100  $\mu$ g) was isolated and digested (sequence and restriction map of scH3k2 see Figure 8) with BbsI/MscI for optimization of L-CDR3, or XhoI/NspV for optimization of H-CDR2. 10  $\mu$ g of purified vector fragments (5 pmole) were ligated with 15 pmole of the L-CDR3 or H-CDR2 library cassettes over night at 16°C. The ligation mixtures were isopropanol precipitated, air-dried, and the pellets were redissolved in 100  $\mu$ l of dd H<sub>2</sub>O. The ligation mixtures were mixed with 1 ml of freshly prepared electrocompetent XL1 Blue cells on ice. Electroporation was performed and the transformants were eluted in SOC medium and shaken at 37°C for 30 minutes. An aliquot was plated out on LB plates (Amp/Tet/Glucose) at 37°C for 6-9



hrs. The number of transformants (library size) was greater than  $10^8$  for both libraries. The libraries were stored as glycerol cultures.

### **7.3. Biopanning**

This was performed as described for the initial H3κ2 H-CDR3 library in Example 2.1. Optimized scFvs binding to FITC could be characterized and analyzed as described in Example 2.2 and 2.3, and further rounds of optimization could be made if necessary.

### References

- Barbas III, C.F., Bain, J.D., Hoekstra, D.M. & Lerner, R.A., PNAS 89, 4457-4461 (1992).
- Better, M., Chang, P., Robinson, R. & Horwitz, A.H., Science 240, 1041-1043 (1988).
- Blake, M.S., Johnston, K.H., Russel-Jones, G.J. & Gotschlich, E.C., Anal. Biochem. 136, 175-179 (1984).
- Carter, P., Kelly, R.F., Rodrigues, M.L., Snedecor, B., Covarrubias, M., Velligan, M.D., Wong, W.L.T., Rowland, A.M., Kotts, C.E., Carver, M.E., Yang, M., Bourell, J.H., Shepard, H.M. & Henner, D., Bio/Technology 10, 163-167 (1992).
- Chothia, C. & Lesk, A.M., J. Biol. Chem. 196, 910-917 (1987).
- Chothia, C., Lesk, A.M., Gherardi, E., Tomlinson, I.A., Walter, G., Marks, J.D., Llewelyn, M.B. & Winter, G., J. Mol. Biol. 227, 799-817 (1992).
- Chothia, C., Lesk, A.M., Tramontano, A., Levitt, M., Smith-Gill, S.J., Air, G., Sheriff, S., Padlan, E.A., Davies, D., Tulip, W.R., Colman, P.M., Spinelli, S., Alzari, P.M. & Poljak, R.J., Nature 342, 877-883 (1989).
- Chuchana, P., Blancher, A., Brockly, F., Alexandre, D., Lefranc, G & Lefranc, M.-P., Eur. J. Immunol. 20, 1317-1325 (1990).
- Cox, J.P.L., Tomlinson, I.M. & Winter, G., Eur. J. Immunol. 24, 827-836 (1994).
- Ge, L., Knappik, A., Pack, P., Freund, C. & Plückthun, A., In: Antibody Engineering. Borrebaeck, C.A.K. (Ed.). p.229-266 (1995), Oxford University Press, New York, Oxford.)
- Gill, S.C. & von Hippel, P.H., Anal. Biochem. 182, 319-326 (1989).
- Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. & Stüber, D., Bio/Technology 6, 1321-1325 (1988).
- Hopp, T.P., Prickett, K.S., Price, V.L., Libby, R.T., March, C.J., Cerretti, D.P., Urdal, D.L. & Conlon, P.J. Bio/Technology 6, 1204-1210 (1988).
- Kabat, E.A., Wu, T.T., Perry, H.M., Gottesmann, K.S. & Foeller, C., Sequences of proteins of immunological interest, NIH publication 91-3242 (1991).
- Knappik, A. & Plückthun, A., Biotechniques 17, 754-761 (1994).
- Knappik, A. & Plückthun, A., Protein Engineering 8, 81-89 (1995).
- Kunkel, T.A., Bebenek, K. & McClary, J., Methods in Enzymol. 204, 125-39 (1991).
- Lindner, P., Guth, B., Wülfing, C., Krebber, C., Steipe, B., Müller, F. & Plückthun, A., Methods: A Companion to Methods Enzymol. 4, 41-56 (1992).
- Lowman, H.B., Bass, S.H., Simpson, N. and Wells, J.A., Biochemistry 30, 10832-10838 (1991).
- Pack, P. & Plückthun, A., Biochemistry 31, 1579-1584 (1992).

- Pack, P., Kujau, M., Schroeckh, V., Knüpfer, U., Wenderoth, R., Riesenborg D. & Plückthun, A., *Bio/Technology* 11, 1271-1277 (1993).
- Pack, P., Ph.D. thesis, Ludwig-Maximilians-Universität München (1994).
- Perlak, F. J., *Nuc. Acids Res.* 18, 7457-7458 (1990).
- Plückthun, A., Krebber, A., Krebber, C., Horn, U., Knüpfer, U., Wenderoth, R., Nieba, L., Proba, K. & Riesenborg, D., A practical approach. *Antibody Engineering* (Ed. J. McCafferty). IRL Press, Oxford, pp. 203-252 (1996).
- Prodromou, C. & Pearl, L.H., *Protein Engineering* 5, 827-829 (1992).
- Rosenberg, S.A. & Lotze, M.T., *Ann. Rev. Immunol.* 4, 681-709 (1986).
- Skerra, A. & Plückthun, A., *Science* 240, 1038-1041 (1988).
- Skerra, A., Pfitzinger, I. & Plückthun, A., *Bio/Technology* 9, 273-278 (1991).
- Sutherland, L., Davidson, J., Glass, L.L., & Jacobs, H.T., *BioTechniques* 18, 458-464, 1995.
- Tomlinson, I.M., Walter, G., Marks, J.D., Llewelyn, M.B. & Winter, G., *J. Mol. Biol.* 227, 776-798 (1992).
- Ullrich, H.D., Patten, P.A., Yang, P.L., Romesberg, F.E. & Schultz, P.G., *Proc. Natl. Acad. Sci. USA* 92, 11907-11911 (1995).
- Van Dijk, K.W., Mortari, F., Kirkham, P.M., Schroeder Jr., H.W. & Milner, E.C.B., *Eur. J. Immunol.* 23, 832-839 (1993).
- Virnekäs, B., Ge, L., Plückthun, A., Schneider, K.C., Wellenhofer, G. & Moroney, S.E., *Nucleic Acids Research* 22, 5600-5607 (1994).
- Vitetta, E.S., Thorpe, P.E. & Uhr, J., *Immunol. Today* 14, 253-259 (1993).
- Williams, S.C. & Winter, G., *Eur. J. Immunol.* 23, 1456-1461 (1993).
- Winter, G., Griffiths, A.D., Hawkins, R.E. & Hoogenboom, H.R., *Ann. Rev. Immunol.* 12, 433-455 (1994).

Table 1A: Human kappa germline gene segments

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
Vk1-1	9	1	O8; O18; DPK1
Vk1-2	1	1	L14; DPK2
Vk1-3	2	1	L15(1); HK101; HK146; HK189
Vk1-4	9	1	L11
Vk1-5	2	1	A30
Vk1-6	1	1	LFVK5
Vk1-7	1	1	LFVK431
Vk1-8	1	1	L1; HK137
Vk1-9	1	1	A20; DPK4
Vk1-10	1	1	L18; Va"
Vk1-11	1	1	L4; L18; Va'; V4a
Vk1-12	2	1	L5; L19(1); Vb; Vb4; DPK5; L19(2); Vb"; DPK6
Vk1-13	2	1	L15(2); HK134; HK166; DPK7
Vk1-14	8	1	L8; Vd; DPK8
Vk1-15	8	1	L9; Ve
Vk1-16	1	1	L12(1); HK102; V1
Vk1-17	2	1	L12(2)
Vk1-18	1	1	O12a (V3b)
Vk1-19	6	1	O2; O12; DPK9
Vk1-20	2	1	L24; Ve"; V13; DPK10
Vk1-21	1	1	O4; O14
Vk1-22	2	1	L22
Vk1-23	2	1	L23
Vk2-1	1	2	A2; DPK12
Vk2-2	6	2	O1; O11(1); DPK13
Vk2-3	6	2	O12(2); V3a
Vk2-4	2	2	L13
Vk2-5	1	2	DPK14
Vk2-6	4	2	A3; A19; DPK15
Vk2-7	4	2	A29; DPK27
Vk2-8	4	2	A13
Vk2-9	1	2	A23

Table 1A: (continued)

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
Vk2-10	4	2	A7; DPK17
Vk2-11	4	2	A17; DPK18
Vk2-12	4	2	A1; DPK19
Vk3-1	11	3	A11; humkv305; DPK20
Vk3-2	1	3	L20; Vg"
Vk3-3	2	3	L2; L16; humkv328; humkv328h2; humkv328h5; DPK21
Vk3-4	11	3	A27; humkv325; VkrF; DPK22
Vk3-5	2	3	L25; DPK23
Vk3-6	2	3	L10(1)
Vk3-7	7	3	L10(2)
Vk3-8	7	3	L6; Vg
Vk4-1	3	4	B3; VklV; DPK24
Vk5-1	10	5	B2; EV15
Vk6-1	12	6	A14; DPK25
Vk6-2	12	6	A10; A26; DPK26
Vk7-1	5	7	B1

Table 1B: Human lambda germline gene segments

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
DPL1	1	1	
DPL2	1	1	HUMLV1L1
DPL3	1	1	HUMLV122
DPL4	1	1	VLAMBDA 1.1
HUMLV117	2	1	
DPL5	1	1	HUMLV117D
DPL6	1	1	
DPL7	1	1	IGLV1S2
DPL8	1	1	HUMLV1042
DPL9	1	1	HUMLV101
DPL10	1	2	
VLAMBDA 2.1	3	2	
DPL11	1	2	
DPL12	1	2	
DPL13	1	2	
DPL14	1	2	
DPL16	1	3	Humlv418; IGLV3S1
DPL23	1	3	VI III.1
Humlv318	4	3	
DPL18	1	7	4A; HUMIGLVA
DPL19	1	7	
DPL21	1	8	VL8.1
HUMLV801	5	8	
DPL22	1	9	
DPL24	1	unassigned	VLAMBDA N.2
gVLX-4.4	6	10	

Table 1C: Human heavy chain germline gene segments

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
VH1-12-1	19	1	DP10; DA-2; DA-6
VH1-12-8	22	1	RR.VH1.2
VH1-12-2	6	1	hv1263
VH1-12-9	7	1	YAC-7; RR.VH1.1; 1-69
VH1-12-3	19	1	DP3
VH1-12-4	19	1	DP21; 4d275a; VH7a
VH1-12-5	18	1	I-4.1b; V1-4.1b
VH1-12-6	21	1	1D37; VH7b ; 7-81; YAC-10
VH1-12-7	19	1	DP14; VH1GRR; V1-18
VH1-13-1	10	1	71-5; DP2
VH1-13-2	10	1	E3-10
VH1-13-3	19	1	DP1
VH1-13-4	12	1	V35
VH1-13-5	8	1	V1-2b
VH1-13-6	18	1	I-2; DP75
VH1-13-7	21	1	V1-2
VH1-13-8	19	1	DP8
VH1-13-9	3	1	1-1
VH1-13-10	19	1	DP12
VH1-13-11	15	1	V13C
VH1-13-12	18	1	I-3b; DP25; V1-3b
VH1-13-13	3	1	1-92
VH1-13-14	18	1	I-3; V1-3
VH1-13-15	19	1	DP15; V1-8
VH1-13-16	3	1	21-2; 3-1; DP7; V1-46
VH1-13-17	16	1	HG3
VH1-13-18	19	1	DP4; 7-2; V1-45
VH1-13-19	27	1	COS 5
VH1-1X-1	19	1	DP5; 1-24P
VH2-21-1	18	2	II-5b
VH2-31-1	2	2	VH2S12-1
VH2-31-2	2	2	VH2S12-7
VH2-31-3	2	2	VH2S12-9; DP27
VH2-31-4	2	2	VH2S12-10
VH2-31-5	14	2	V2-26; DP26; 2-26
VH2-31-6	15	2	VF2-26

Table 1C: (continued)

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
VH2-31-7	19	2	DP28; DA-7
VH2-31-14	7	2	YAC-3; 2-70
VH2-31-8	2	2	VH2S12-5
VH2-31-9	2	2	VH2S12-12
VH2-31-10	18	2	II-5; V2-5
VH2-31-11	2	2	VH2S12-2; VH2S12-8
VH2-31-12	2	2	VH2S12-4; VH2S12-6
VH2-31-13	2	2	VH2S12-14
VH3-11-1	13	3	v65-2; DP44
VH3-11-2	19	3	DP45
VH3-11-3	3	3	13-2; DP48
VH3-11-4	19	3	DP52
VH3-11-5	14	3	v3-13
VH3-11-6	19	3	DP42
VH3-11-7	3	3	8-1B; YAC-5; 3-66
VH3-11-8	14	3	V3-53
VH3-13-1	3	3	22-2B; DP35; V3-11
VH3-13-5	19	3	DP59; VH19; V3-35
VH3-13-6	25	3	f1-p1; DP61
VH3-13-7	19	3	DP46; GL-SJ2; COS 8; hv3005; hv3005f3; 3d21b; 56p1
VH3-13-8	24	3	VH26
VH3-13-9	5	3	vh26c
VH3-13-10	19	3	DP47; VH26; 3-23
VH3-13-11	3	3	1-91
VH3-13-12	19	3	DP58
VH3-13-13	3	3	1-9III; DP49; 3-30; 3d28.1
VH3-13-14	24	3	3019B9; DP50; 3-33; 3d277
VH3-13-15	27	3	COS 3
VH3-13-16	19	3	DP51
VH3-13-17	16	3	H11
VH3-13-18	19	3	DP53; COS 6; 3-74; DA-8
VH3-13-19	19	3	DP54; VH3-11; V3-7
VH3-13-20	14	3	V3-64; YAC-6
VH3-13-21	14	3	V3-48
VH3-13-22	14	3	V3-43; DP33
VH3-13-23	14	3	V3-33



Table 1C: (continued)

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
VH3-13-24	14	3	V3-21; DP77
VH3-13-25	14	3	V3-20; DP32
VH3-13-26	14	3	V3-9; DP31
VH3-14-1	3	3	12-2; DP29; 3-72; DA-3
VH3-14-4	7	3	YAC-9; 3-73; MTGL
VH3-14-2	4	3	VHD26
VH3-14-3	19	3	DP30
VH3-1X-1	1	3	LSG8.1; LSG9.1; LSG10.1; HUM12IGVH; HUM13IGVH
VH3-1X-2	1	3	LSG11.1; HUM4IGVH
VH3-1X-3	3	3	9-1; DP38; LSG7.1; RCG1.1; LSG1.1; LSG3.1; LSG5.1; HUM15IGVH; HUM2IGVH; HUM9IGVH
VH3-1X-4	1	3	LSG4.1
VH3-1X-5	1	3	LSG2.1
VH3-1X-6	1	3	LSG6.1; HUM10IGVH
VH3-1X-7	18	3	3-15; V3-15
VH3-1X-8	1	3	LSG12.1; HUM5IGVH
VH3-1X-9	14	3	V3-49
VH4-11-1	22	4	Tou-VH4.21
VH4-11-2	17	4	VH4.21; DP63; VH5; 4d76; V4-34
VH4-11-3	23	4	4.44
VH4-11-4	23	4	4.44.3
VH4-11-5	23	4	4.36
VH4-11-6	23	4	4.37
VH4-11-7	18	4	IV-4; 4.35; V4-4
VH4-11-8	17	4	VH4.11; 3d197d; DP71; 58p2
VH4-11-9	20	4	H7
VH4-11-10	20	4	H8
VH4-11-11	20	4	H9
VH4-11-12	17	4	VH4.16
VH4-11-13	23	4	4.38
VH4-11-14	17	4	VH4.15
VH4-11-15	11	4	58
VH4-11-16	10	4	71-4; V4-59
VH4-21-1	11	4	11
VH4-21-2	17	4	VH4.17; VH4.23; 4d255; 4.40; DP69
VH4-21-3	17	4	VH4.19; 79; V4-4b

Table 1C: (continued)

Used Name <sup>1</sup>	Reference <sup>2</sup>	Family <sup>3</sup>	Germline genes <sup>4</sup>
VH4-21-4	19	4	DP70; 4d68; 4.41
VH4-21-5	19	4	DP67; VH4-4B
VH4-21-6	17	4	VH4.22; VHSP; VH-JA
VH4-21-7	17	4	VH4.13; 1-9II; 12G-1; 3d28d; 4.42; DP68; 4-28
VH4-21-8	26	4	hv4005; 3d24d
VH4-21-9	17	4	VH4.14
VH4-31-1	23	4	4.34; 3d230d; DP78
VH4-31-2	23	4	4.34.2
VH4-31-3	19	4	DP64; 3d216d
VH4-31-4	19	4	DP65; 4-31; 3d277d
VH4-31-5	23	4	4.33; 3d75d
VH4-31-6	20	4	H10
VH4-31-7	20	4	H11
VH4-31-8	23	4	4.31
VH4-31-9	23	4	4.32
VH4-31-10	20	4	3d277d
VH4-31-11	20	4	3d216d
VH4-31-12	20	4	3d279d
VH4-31-13	17	4	VH4.18; 4d154; DP79
VH4-31-14	8	4	V4-39
VH4-31-15	11	4	2-1; DP79
VH4-31-16	23	4	4.30
VH4-31-17	17	4	VH4.12
VH4-31-18	10	4	71-2; DP66
VH4-31-19	23	4	4.39
VH4-31-20	8	4	V4-61
VH5-12-1	9	5	VH251; DP73; VHVCW; 51-R1; VHVLB; VHVCB; VHVTT; VHVAU; VHVBLK; VhAU; V5-51
VH5-12-2	17	5	VHVJB
VH5-12-3	3	5	1-v; DP80; 5-78
VH5-12-4	9	5	VH32; VHVRG; VHVMW; 5-2R1
VH6-35-1	4	6	VHVI; VH6; VHVIIS; VHVITE; VHVIJB; VHVICH; VHVICW; VHVIBLK; VHVIMW; DP74; 6-1G1; V6-1

Table 2A: rearranged human kappa sequences

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
III-3R	108	1	O8	1	1,1%	70
No.86	109	1	O8	3	3,2%	80
AU	108	1	O8	6	6,3%	103
ROY	108	1	O8	6	6,3%	43
IC4	108	1	O8	6	6,3%	70
HIV-B26	106	1	O8	3	3,2%	8
GRI	108	1	O8	8	8,4%	30
AG	106	1	O8	8	8,6%	116
REI	108	1	O8	9	9,5%	86
CLL PATIENT 16	88	1	O8	2	2,3%	122
CLL PATIENT 14	87	1	O8	2	2,3%	122
CLL PATIENT 15	88	1	O8	2	2,3%	122
GM4672	108	1	O8	11	11,6%	24
HUM. YFC51.1	108	1	O8	12	12,6%	110
LAY	108	1	O8	12	12,6%	48
HIV-b13	106	1	O8	9	9,7%	8
MAL-NaCl	108	1	O8	13	13,7%	102
STRAb SA-1A	108	1	O2	0	0,0%	120
HuVHCAMP	108	1	O8	13	13,7%	100
CRO	108	1	O2	10	10,5%	30
Am107	108	1	O2	12	12,6%	108
WALKER	107	1	O2	4	4,2%	57
III-2R	109	1	A20	0	0,0%	70
FOG1-A4	107	1	A20	4	4,2%	41
HK137	95	1	L1	0	0,0%	10
CEA4-8A	107	1	O2	7	7,4%	41
Va'	95	1	L4	0	0,0%	90
TR1.21	108	1	O2	4	4,2%	92
HAU	108	1	O2	6	6,3%	123
HK102	95	1	L12(1)	0	0,0%	9
H20C3K	108	1	L12(2)	3	3,2%	125
CHEB	108	1	O2	7	7,4%	5
HK134	95	1	L15(2)	0	0,0%	10
TEL9	108	1	O2	9	9,5%	73

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
TR1.32	103	1	O2	3	3,2%	92
RF-KES1	97	1	A20	4	4,2%	121
WES	108	1	L5	10	10,5%	61
DILp1	95	1	O4	1	1,1%	70
SA-4B	107	1	L12(2)	8	8,4%	120
HK101	95	1	L15(1)	0	0,0%	9
TR1.23	108	1	O2	5	5,3%	92
HF2-1/17	108	1	A30	0	0,0%	4
2E7	108	1	A30	1	1,1%	62
33.C9	107	1	L12(2)	7	7,4%	126
3D6	105	1	L12(2)	2	2,1%	34
I-2a	108	1	L8	8	8,4%	70
RF-KL1	97	1	L8	4	4,2%	121
TNF-E7	108	1	A30	9	9,5%	41
TR1.22	108	1	O2	7	7,4%	92
HIV-B35	106	1	O2	2	2,2%	8
HIV-b22	106	1	O2	2	2,2%	8
HIV-b27	106	1	O2	2	2,2%	8
HIV-B8	107	1	O2	10	10,8%	8
HIV-b8	107	1	O2	10	10,8%	8
RF-SJ5	95	1	A30	5	5,3%	113
GAL(I)	108	1	A30	6	6,3%	64
R3.5H5G	108	1	O2	6	6,3%	70
HIV-b14	106	1	A20	2	2,2%	8
TNF-E1	105	1	L5	8	8,4%	41
WEA	108	1	A30	8	8,4%	37
EU	108	1	L12(2)	5	5,3%	40
FOG1-G8	108	1	L8	11	11,6%	41
1X7RG1	108	1	L1	8	8,4%	70
BLI	108	1	L8	3	3,2%	72
KUE	108	1	L12(2)	11	11,6%	32
LUNm01	108	1	L12(2)	10	10,5%	6
HIV-b1	106	1	A20	4	4,3%	8
HIV-s4	103	1	O2	2	2,2%	8

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
CAR	107	1	L12(2)	11	11,7%	79
BR	107	1	L12(2)	11	11,6%	50
CLL PATIENT 10	88	1	O2	0	0,0%	122
CLL PATIENT 12	88	1	O2	0	0,0%	122
KING	108	1	L12(2)	12	12,6%	30
V13	95	1	L24	0	0,0%	46
CLL PATIENT 11	87	1	O2	0	0,0%	122
CLL PATIENT 13	87	1	O2	0	0,0%	122
CLL PATIENT 9	88	1	O12	1	1,1%	122
HIV-B2	106	1	A20	9	9,7%	8
HIV-b2	106	1	A20	9	9,7%	8
CLL PATIENT 5	88	1	A20	1	1,1%	122
CLL PATIENT 1	88	1	L8	2	2,3%	122
CLL PATIENT 2	88	1	L8	0	0,0%	122
CLL PATIENT 7	88	1	L5	0	0,0%	122
CLL PATIENT 8	88	1	L5	0	0,0%	122
HIV-b5	105	1	L5	11	12,0%	8
CLL PATIENT 3	87	1	L8	1	1,1%	122
CLL PATIENT 4	88	1	L9	0	0,0%	122
CLL PATIENT 18	85	1	L9	6	7,1%	122
CLL PATIENT 17	86	1	L12(2)	7	8,1%	122
HIV-b20	107	3	A27	11	11,7%	8
2C12	108	1	L12(2)	20	21,1%	68
1B11	108	1	L12(2)	20	21,1%	68
1H1	108	1	L12(2)	21	22,1%	68
2A12	108	1	L12(2)	21	22,1%	68
CUR	109	3	A27	0	0,0%	66
GLO	109	3	A27	0	0,0%	16
RF-TS1	96	3	A27	0	0,0%	121
GAR'	109	3	A27	0	0,0%	67
FLO	109	3	A27	0	0,0%	66
PIE	109	3	A27	0	0,0%	91
HAH 14.1	109	3	A27	1	1,0%	51
HAH 14.2	109	3	A27	1	1,0%	51

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
HAH 16.1	109	3	A27	1	1,0%	51
NOV	109	3	A27	1	1,0%	52
33.F12	108	3	A27	1	1,0%	126
8E10	110	3	A27	1	1,0%	25
TH3	109	3	A27	1	1,0%	25
HIC (R)	108	3	A27	0	0,0%	51
SON	110	3	A27	1	1,0%	67
PAY	109	3	A27	1	1,0%	66
GOT	109	3	A27	1	1,0%	67
mAbA6H4C5	109	3	A27	1	1,0%	12
BOR'	109	3	A27	2	2,1%	84
RF-SJ3	96	3	A27	2	2,1%	121
SIE	109	3	A27	2	2,1%	15
ESC	109	3	A27	2	2,1%	98
HEW'	110	3	A27	2	2,1%	98
YES8c	109	3	A27	3	3,1%	33
TI	109	3	A27	3	3,1%	114
mAb113	109	3	A27	3	3,1%	71
HEW	107	3	A27	0	0,0%	94
BRO	106	3	A27	0	0,0%	94
ROB	106	3	A27	0	0,0%	94
NG9	96	3	A27	4	4,2%	11
NEU	109	3	A27	4	4,2%	66
WOL	109	3	A27	4	4,2%	2
35G6	109	3	A27	4	4,2%	59
RF-SJ4	109	3	A11	0	0,0%	88
KAS	109	3	A27	4	4,2%	84
BRA	106	3	A27	1	1,1%	94
HAH	106	3	A27	1	1,1%	94
HIC	105	3	A27	0	0,0%	94
FS-2	109	3	A27	6	6,3%	87
JH'	107	3	A27	6	6,3%	38
EV1-15	109	3	A27	6	6,3%	83
SCA	108	3	A27	6	6,3%	65

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Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
mAb112	109	3	A27	6	6,3%	71
SIC	103	3	A27	3	3,3%	94
SA-4A	109	3	A27	6	6,3%	120
SER	108	3	A27	6	6,3%	98
GOL	109	3	A27	7	7,3%	82
B5G10K	105	3	A27	9	9,7%	125
HG2B10K	110	3	A27	-9	-9,4%	125
Taykv322	105	3	A27	5	5,4%	52
CLL PATIENT 24	89	3	A27	1	1,1%	122
HIV-b24	107	3	A27	7	7,4%	8
HIV-b6	107	3	A27	7	7,4%	8
Taykv310	99	3	A27	1	1,1%	52
KA3D1	108	3	L6	0	0,0%	85
19.E7	107	3	L6	0	0,0%	126
rsv6L	109	3	A27	12	12,5%	7
Taykv320	98	3	A27	1	1,2%	52
Vh	96	3	L10(2)	0	0,0%	89
LS8	108	3	L6	1	1,1%	109
LS1	108	3	L6	1	1,1%	109
LS2S3-3	107	3	L6	2	2,1%	99
LS2	108	3	L6	1	1,1%	109
LS7	108	3	L6	1	1,1%	109
LS2S3-4d	107	3	L6	2	2,1%	99
LS2S3-4a	107	3	L6	2	2,1%	99
LS4	108	3	L6	1	1,1%	109
LS6	108	3	L6	1	1,1%	109
LS2S3-10a	107	3	L6	2	2,1%	99
LS2S3-8c	107	3	L6	2	2,1%	99
LS5	108	3	L6	1	1,1%	109
LS2S3-5	107	3	L6	3	3,2%	99
LUNm03	109	3	A27	13	13,5%	6
IARC/BL41	108	3	A27	13	13,7%	55
slkv22	99	3	A27	3	3,5%	13
POP	108	3	L6	4	4,2%	111

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
LS2S3-10b	107	3	L6	3	3,2%	99
LS2S3-8f	107	3	L6	3	3,2%	99
LS2S3-12	107	3	L6	3	3,2%	99
HIV-B30	107	3	A27	11	11,7%	8
HIV-B20	107	3	A27	11	11,7%	8
HIV-b3	108	3	A27	11	11,7%	8
HIV-s6	104	3	A27	9	9,9%	8
YSE	107	3	L2/L16	1	1,1%	72
POM	109	3	L2/L16	9	9,4%	53
Humkv328	95	3	L2/L16	1	1,1%	19
CLL	109	3	L2/L16	3	3,2%	47
LES	96	3	L2/L16	3	3,2%	38
HIV-s5	104	3	A27	11	12,1%	8
HIV-s7	104	3	A27	11	12,1%	8
slkv1	99	3	A27	7	8,1%	13
Humka31es	95	3	L2/L16	4	4,2%	18
slkv12	101	3	A27	8	9,2%	13
RF-TS2	95	3	L2/L16	3	3,2%	121
II-1	109	3	L2/L16	4	4,2%	70
HIV-s3	105	3	A27	13	14,3%	8
RF-TMC1	96	3	L6	10	10,5%	121
GER	109	3	L2/L16	7	7,4%	75
GF4/1.1	109	3	L2/L16	8	8,4%	36
mAb114	109	3	L2/L16	6	6,3%	71
HIV-loop13	109	3	L2/L16	7	7,4%	8
bkv16	86	3	L6	1	1,2%	13
CLL PATIENT 29	86	3	L6	1	1,2%	122
slkv9	98	3	L6	3	3,5%	13
bkv17	99	3	L6	1	1,2%	13
slkv14	99	3	L6	1	1,2%	13
slkv16	101	3	L6	2	2,3%	13
bkv33	101	3	L6	4	4,7%	13
slkv15	99	3	L6	2	2,3%	13
bkv6	100	3	L6	3	3,5%	13



Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
R6B8K	108	3	L2/L16	12	12,6%	125
AL 700	107	3	L2/L16	9	9,5%	117
slkv11	100	3	L2/L16	3	3,5%	13
slkv4	97	3	L6	4	4,8%	13
CLL PATIENT 26	87	3	L2/L16	1	1,1%	122
AL Se124	103	3	L2/L16	9	9,5%	117
slkv13	100	3	L2/L16	6	7,0%	13
bkv7	100	3	L2/L16	5	5,8%	13
bkv22	100	3	L2/L16	6	7,0%	13
CLL PATIENT 27	84	3	L2/L16	0	0,0%	122
bkv35	100	3	L6	8	9,3%	13
CLL PATIENT 25	87	3	L2/L16	4	4,6%	122
slkv3	86	3	L2/L16	7	8,1%	13
slkv7	99	1	O2	7	8,1%	13
HuFd79	111	3	L2/L16	24	24,2%	21
RAD	99	3	A27	9	10,3%	78
CLL PATIENT 28	83	3	L2/L16	4	4,8%	122
REE	104	3	L2/L16	25	27,2%	95
FR4	99	3	A27	8	9,2%	77
MD3.3	92	3	L6	1	1,3%	54
MD3.1	92	3	L6	0	0,0%	54
GA3.6	92	3	L6	2	2,6%	54
M3.5N	92	3	L6	3	3,8%	54
WEI'	82	3	A27	0	0,0%	65
MD3.4	92	3	L2/L16	1	1,3%	54
MD3.2	91	3	L6	3	3,8%	54
VER	97	3	A27	19	22,4%	20
CLL PATIENT 30	78	3	L6	3	3,8%	122
M3.1N	92	3	L2/L16	1	1,3%	54
MD3.6	91	3	L2/L16	0	0,0%	54
MD3.8	91	3	L2/L16	0	0,0%	54
GA3.4	92	3	L6	7	9,0%	54
M3.6N	92	3	A27	0	0,0%	54
MD3.10	92	3	A27	0	0,0%	54

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
MD3.13	91	3	A27	0	0,0%	54
MD3.7	93	3	A27	0	0,0%	54
MD3.9	93	3	A27	0	0,0%	54
GA3.1	93	3	A27	6	7,6%	54
bkv32	101	3	A27	5	5,7%	13
GA3.5	93	3	A27	5	6,3%	54
GA3.7	92	3	A27	7	8,9%	54
MD3.12	92	3	A27	2	2,5%	54
M3.2N	90	3	L6	6	7,8%	54
MD3.5	92	3	A27	1	1,3%	54
M3.4N	91	3	L2/L16	8	10,3%	54
M3.8N	91	3	L2/L16	7	9,0%	54
M3.7N	92	3	A27	3	3,8%	54
GA3.2	92	3	A27	9	11,4%	54
GA3.8	93	3	A27	4	5,1%	54
GA3.3	92	3	A27	8	10,1%	54
M3.3N	92	3	A27	5	6,3%	54
B6	83	3	A27	8	11,3%	78
E29.1 KAPPA	78	3	L2/L16	0	0,0%	22
SCW	108	1	O8	12	12,6%	31
REI-based CAMPATH-9	107	1	O8	14	14,7%	39
RZ	107	1	O8	14	14,7%	50
BI	108	1	O8	14	14,7%	14
AND	107	1	O2	13	13,7%	69
2A4	109	1	O2	12	12,6%	23
KA	108	1	O8	19	20,0%	107
MEV	109	1	O2	14	14,7%	29
DEE	106	1	O2	13	14,0%	76
OU(IOC)	108	1	O2	18	18,9%	60
HuRSV19VK	111	1	O8	21	21,0%	115
SP2	108	1	O2	17	17,9%	93
BJ26	99	1	O8	21	24,1%	1
NI	112	1	O8	24	24,2%	106
BMA 0310EUCIV2	106	1	L12(1)	21	22,3%	105

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Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
CLL PATIENT 6	71	1	A20	0	0,0%	122
BJ19	85	1	O8	16	21,9%	1
GM 607	113	2	A3	0	0,0%	58
R5A3K	114	2	A3	1	1,0%	125
R1C8K	114	2	A3	1	1,0%	125
VK2.R149	113	2	A3	2	2,0%	118
TR1.6	109	2	A3	4	4,0%	92
TR1.37	104	2	A3	5	5,0%	92
FS-1	113	2	A3	6	6,0%	87
TR1.8	110	2	A3	6	6,0%	92
NIM	113	2	A3	8	8,0%	28
Inc	112	2	A3	11	11,0%	35
TEW	107	2	A3	6	6,4%	96
CUM	114	2	O1	7	6,9%	44
HRF1	71	2	A3	4	5,6%	124
CLL PATIENT 19	87	2	A3	0	0,0%	122
CLL PATIENT 20	87	2	A3	0	0,0%	122
MIL	112	2	A3	16	16,2%	26
FR	113	2	A3	20	20,0%	101
MAL-Urine	83	1	O2	6	8,6%	102
Taykv306	73	3	A27	1	1,6%	52
Taykv312	75	3	A27	1	1,6%	52
HIV-b29	93	3	A27	14	17,5%	8
1-185-37	110	3	A27	0	0,0%	119
1-187-29	110	3	A27	0	0,0%	119
TT117	110	3	A27	9	9,4%	63
HIV-loop8	108	3	A27	16	16,8%	8
rsv23L	108	3	A27	16	16,8%	7
HIV-b7	107	3	A27	14	14,9%	8
HIV-b11	107	3	A27	15	16,0%	8
HIV-LC1	107	3	A27	19	20,2%	8
HIV-LC7	107	3	A27	20	21,3%	8
HIV-LC22	107	3	A27	21	22,3%	8
HIV-LC13	107	3	A27	21	22,3%	8

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Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
HIV-LC3	107	3	A27	21	22,3%	8
HIV-LC5	107	3	A27	21	22,3%	8
HIV-LC28	107	3	A27	21	22,3%	8
HIV-b4	107	3	A27	22	23,4%	8
CLL PATIENT 31	87	3	A27	15	17,2%	122
HIV-loop2	108	3	L2/L16	17	17,9%	8
HIV-loop35	108	3	L2/L16	17	17,9%	8
HIV-LC11	107	3	A27	23	24,5%	8
HIV-LC24	107	3	A27	23	24,5%	8
HIV-b12	107	3	A27	24	25,5%	8
HIV-LC25	107	3	A27	24	25,5%	8
HIV-b21	107	3	A27	24	25,5%	8
HIV-LC26	107	3	A27	26	27,7%	8
G3D10K	108	1	L12(2)	12	12,6%	125
TT125	108	1	L5	8	8,4%	63
HIV-s2	103	3	A27	28	31,1%	8
265-695	108	1	L5	7	7,4%	3
2-115-19	108	1	A30	2	2,1%	119
rsv13L	107	1	O2	20	21,1%	7
HIV-b18	106	1	O2	14	15,1%	8
RF-KL5	98	3	L6	36	36,7%	97
ZM1-1	113	2	A17	7	7,0%	3
HIV-s8	103	1	O8	16	17,8%	8
K- EV15	95	5	B2	0	0,0%	112
RF-TS3	100	2	A23	0	0,0%	121
HF-21/28	111	2	A17	1	1,0%	17
RPMI6410	113	2	A17	1	1,0%	42
JC11	113	2	A17	1	1,0%	49
O-81	114	2	A17	5	5,0%	45
FK-001	113	4	B3	0	0,0%	81
CD5+.28	101	4	B3	1	1,0%	27
LEN	114	4	B3	1	1,0%	104
UC	114	4	B3	1	1,0%	111
CD5+.5	101	4	B3	1	1,0%	27

Table 2A: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
CD5+.26	101	4	B3	1	1,0%	27
CD5+.12	101	4	B3	2	2,0%	27
CD5+.23	101	4	B3	2	2,0%	27
CD5+.7	101	4	B3	2	2,0%	27
VJI	113	4	B3	3	3,0%	56
LOC	113	4	B3	3	3,0%	72
MAL	113	4	B3	3	3,0%	72
CD5+.6	101	4	B3	3	3,0%	27
H2F	113	4	B3	3	3,0%	70
PB17IV	114	4	B3	4	4,0%	74
CD5+.27	101	4	B3	4	4,0%	27
CD5+.9	101	4	B3	4	4,0%	27
CD5-.28	101	4	B3	5	5,0%	27
CD5-.26	101	4	B3	6	5,9%	27
CD5+.24	101	4	B3	6	5,9%	27
CD5+.10	101	4	B3	6	5,9%	27
CD5-.19	101	4	B3	6	5,9%	27
CD5-.18	101	4	B3	7	6,9%	27
CD5-.16	101	4	B3	8	7,9%	27
CD5-.24	101	4	B3	8	7,9%	27
CD5-.17	101	4	B3	10	9,9%	27
MD4.1	92	4	B3	0	0,0%	54
MD4.4	92	4	B3	0	0,0%	54
MD4.5	92	4	B3	0	0,0%	54
MD4.6	92	4	B3	0	0,0%	54
MD4.7	92	4	B3	0	0,0%	54
MD4.2	92	4	B3	1	1,3%	54
MD4.3	92	4	B3	5	6,3%	54
CLL PATIENT 22	87	2	A17	2	2,3%	122
CLL PATIENT 23	84	2	A17	2	2,4%	122

Table 2B: rearranged human lambda sequences

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
WAH	110	1	DPL3	7	7%	68
1B9/F2	112	1	DPL3	7	7%	9
DIA	112	1	DPL2	7	7%	36
mAb67	89	1	DPL3	0	0%	29
HiH2	110	1	DPL3	12	11%	3
NIG-77	112	1	DPL2	9	9%	72
OKA	112	1	DPL2	7	7%	84
KOL	112	1	DPL2	12	11%	40
T2:C5	111	1	DPL5	0	0%	6
T2:C14	110	1	DPL5	0	0%	6
PR-TS1	110	1	DPL5	0	0%	55
4G12	111	1	DPL5	1	1%	35
KIM46L	112	1	HUMLV117	0	0%	8
Fog-B	111	1	DPL5	3	3%	31
9F2L	111	1	DPL5	3	3%	79
mAb111	110	1	DPL5	3	3%	48
PHOX15	111	1	DPL5	4	4%	49
BL2	111	1	DPL5	4	4%	74
NIG-64	111	1	DPL5	4	4%	72
RF-SJ2	100	1	DPL5	6	6%	78
AL EZI	112	1	DPL5	7	7%	41
ZIM	112	1	HUMLV117	7	7%	18
RF-SJ1	100	1	DPL5	9	9%	78
IGLV1.1	98	1	DPL4	0	0%	1
NEW	112	1	HUMLV117	11	10%	42
CB-201	87	1	DPL2	1	1%	62
MEM	109	1	DPL2	6	6%	50
H210	111	2	DPL10	4	4%	45
NOV	110	2	DPL10	8	8%	25
NEI	111	2	DPL10	8	8%	24
AL MC	110	2	DPL11	6	6%	28
MES	112	2	DPL11	8	8%	84
FOG1-A3	111	2	DPL11	9	9%	27
AL NOV	112	2	DPL11	7	7%	28

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Table 2B: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
HMST-1	110	2	DPL11	4	4%	82
HBW4-1	108	2	DPL12	9	9%	52
WH	110	2	DPL11	11	11%	34
11-50	110	2	DPL11	7	7%	82
HBp2	110	2	DPL12	8	8%	3
NIG-84	113	2	DPL11	12	11%	73
VIL	112	2	DPL11	9	9%	58
TRO	111	2	DPL12	10	10%	61
ES492	108	2	DPL11	15	15%	76
mAb216	89	2	DPL12	1	1%	7
BSA3	109	3	DPL16	0	0%	49
THY-29	110	3	DPL16	0	0%	27
PR-TS2	108	3	DPL16	0	0%	55
E29.1 LAMBDA	107	3	DPL16	1	1%	13
mAb63	109	3	DPL16	2	2%	29
TEL14	110	3	DPL16	6	6%	49
6H-3C4	108	3	DPL16	7	7%	39
SH	109	3	DPL16	7	7%	70
AL GIL	109	3	DPL16	8	8%	23
H6-3C4	108	3	DPL16	8	8%	83
V-lambda-2.DS	111	2	DPL11	3	3%	15
8.12 ID	110	2	DPL11	3	3%	81
DSC	111	2	DPL11	3	3%	56
PV11	110	2	DPL11	1	1%	56
33.H11	110	2	DPL11	4	4%	81
AS17	111	2	DPL11	7	7%	56
SD6	110	2	DPL11	7	7%	56
KS3	110	2	DPL11	9	9%	56
PV6	110	2	DPL12	5	5%	56
NGD9	110	2	DPL11	7	7%	56
MUC1-1	111	2	DPL11	11	10%	27
A30c	111	2	DPL10	6	6%	56
KS6	110	2	DPL12	6	6%	56
TEL13	111	2	DPL11	11	10%	49

Table 2B: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
AS7	110	2	DPL12	6	6%	56
MCG	112	2	DPL12	12	11%	20
U266L	110	2	DPL12	13	12%	77
PR-SJ2	110	2	DPL12	14	13%	55
BOH	112	2	DPL12	11	10%	37
TOG	111	2	DPL11	19	18%	53
TEL16	111	2	DPL11	19	18%	49
No.13	110	2	DPL10	14	13%	52
BO	112	2	DPL12	18	17%	80
WIN	112	2	DPL12	17	16%	11
BUR	104	2	DPL12	15	15%	46
NIG-58	110	2	DPL12	20	19%	69
WEIR	112	2	DPL11	26	25%	21
THY-32	111	1	DPL8	8	8%	27
TNF-H9G1	111	1	DPL8	9	9%	27
mAb61	111	1	DPL3	1	1%	29
LV1L1	98	1	DPL2	0	0%	54
HA	113	1	DPL3	14	13%	63
LA1L1	111	1	DPL2	3	3%	54
RHE	112	1	DPL1	17	16%	22
K1B12L	113	1	DPL8	17	16%	79
LOC	113	1	DPL2	15	14%	84
NIG-51	112	1	DPL2	12	11%	67
NEWM	104	1	DPL8	23	22%	10
MD3-4	106	3	DPL23	14	13%	4
COX	112	1	DPL2	13	12%	84
HiH10	106	3	DPL23	13	12%	3
VOR	112	1	DPL2	16	15%	16
AL POL	113	1	DPL2	16	15%	57
CD4-74	111	1	DPL2	19	18%	27
AMYLOID MOL	102	3	DPL23	15	15%	30
OST577	108	3	Humlv318	10	10%	4
NIG-48	113	1	DPL3	42	40%	66
CARR	108	3	DPL23	18	17%	19

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Table 2B: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
mAb60	108	3	DPL23	14	13%	29
NIG-68	99	3	DPL23	25	26%	32
KERN	107	3	DPL23	26	25%	59
ANT	106	3	DPL23	17	16%	19
LEE	110	3	DPL23	18	17%	85
CLE	94	3	DPL23	17	17%	19
VL8	98	8	DPL21	0	0%	81
MOT	110	3	Humlv318	23	22%	38
GAR	108	3	DPL23	26	25%	33
32.B9	98	8	DPL21	5	5%	81
PUG	108	3	Humlv318	24	23%	19
T1	115	8	HUMLV801	52	50%	6
RF-TS7	96	7	DPL18	4	4%	60
YM-1	116	8	HUMLV801	51	49%	75
K6H6	112	8	HUMLV801	20	19%	44
K5C7	112	8	HUMLV801	20	19%	44
K5B8	112	8	HUMLV801	20	19%	44
K5G5	112	8	HUMLV801	20	19%	44
K4B8	112	8	HUMLV801	19	18%	44
K6F5	112	8	HUMLV801	17	16%	44
HIL	108	3	DPL23	22	21%	47
KIR	109	3	DPL23	20	19%	19
CAP	109	3	DPL23	19	18%	84
1B8	110	3	DPL23	22	21%	43
SHO	108	3	DPL23	19	18%	19
HAN	108	3	DPL23	20	19%	19
cML23	96	3	DPL23	3	3%	12
PR-SJ1	96	3	DPL23	7	7%	55
BAU	107	3	DPL23	9	9%	5
TEX	99	3	DPL23	8	8%	19
X(PET)	107	3	DPL23	9	9%	51
DOY	106	3	DPL23	9	9%	19
COT	106	3	DPL23	13	12%	19
Pag-1	111	3	Humlv318	5	5%	31

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Table 2B: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
DIS	107	3	HuMlv318	2	2%	19
WIT	108	3	HuMlv318	7	7%	19
LRH	108	3	HuMlv318	12	11%	19
S1-1	108	3	HuMlv318	12	11%	52
DEL	108	3	HuMlv318	14	13%	17
TYR	108	3	HuMlv318	11	10%	19
J.RH	109	3	HuMlv318	13	12%	19
THO	112	2	DPL13	38	36%	26
LBV	113	1	DPL3	38	36%	2
WLT	112	1	DPL3	33	31%	14
SUT	112	2	DPL12	37	35%	65

Table 2C: rearranged human heavy chain sequences

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
21/28	119	1	VH1-13-12	0	0,0%	31
8E10	123	1	VH1-13-12	0	0,0%	31
MUC1-1	118	1	VH1-13-6	4	4,1%	42
gF1	98	1	VH1-13-12	10	10,2%	75
VHGL 1.2	98	1	VH1-13-6	2	2,0%	26
HV1L1	98	1	VH1-13-6	0	0,0%	81
RF-TS7	104	1	VH1-13-6	3	3,1%	96
E55 1.A15	106	1	VH1-13-15	1	1,0%	26
HA1L1	126	1	VH1-13-6	7	7,1%	81
UC	123	1	VH1-13-6	5	5,1%	115
WIL2	123	1	VH1-13-6	6	6,1%	55
R3.5H5G	122	1	VH1-13-6	10	10,2%	70
N89P2	123	1	VH1-13-16	11	11,2%	77
mAb113	126	1	VH1-13-6	10	10,2%	71
LS2S3-3	125	1	VH1-12-7	5	5,1%	98
LS2S3-12a	125	1	VH1-12-7	5	5,1%	98
LS2S3-5	125	1	VH1-12-7	5	5,1%	98
LS2S3-12e	125	1	VH1-12-7	5	5,1%	98
LS2S3-4	125	1	VH1-12-7	5	5,1%	98
LS2S3-10	125	1	VH1-12-7	5	5,1%	98
LS2S3-12d	125	1	VH1-12-7	6	6,1%	98
LS2S3-8	125	1	VH1-12-7	5	5,1%	98
LS2	125	1	VH1-12-7	6	6,1%	113
LS4	105	1	VH1-12-7	6	6,1%	113
LS5	125	1	VH1-12-7	6	6,1%	113
LS1	125	1	VH1-12-7	6	6,1%	113
LS6	125	1	VH1-12-7	6	6,1%	113
LS8	125	1	VH1-12-7	7	7,1%	113
THY-29	122	1	VH1-12-7	0	0,0%	42
1B9/F2	122	1	VH1-12-7	10	10,2%	21
51P1	122	1	VH1-12-1	0	0,0%	105
NEI	127	1	VH1-12-1	0	0,0%	55
AND	127	1	VH1-12-1	0	0,0%	55
L7	127	1	VH1-12-1	0	0,0%	54
L22	124	1	VH1-12-1	0	0,0%	54
L24	127	1	VH1-12-1	0	0,0%	54

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
L26	116	1	VH1-12-1	0	0,0%	54
L33	119	1	VH1-12-1	0	0,0%	54
L34	117	1	VH1-12-1	0	0,0%	54
L36	118	1	VH1-12-1	0	0,0%	54
L39	120	1	VH1-12-1	0	0,0%	54
L41	120	1	VH1-12-1	0	0,0%	54
L42	125	1	VH1-12-1	0	0,0%	54
VHGL 1.8	101	1	VH1-12-1	0	0,0%	26
783c	127	1	VH1-12-1	0	0,0%	22
X17115	127	1	VH1-12-1	0	0,0%	37
L25	124	1	VH1-12-1	0	0,0%	54
L17	120	1	VH1-12-1	1	1,0%	54
L30	127	1	VH1-12-1	1	1,0%	54
L37	120	1	VH1-12-1	1	1,0%	54
TNF-E7	116	1	VH1-12-1	2	2,0%	42
mAb111	122	1	VH1-12-1	7	7,1%	71
III-2R	122	1	VH1-12-9	3	3,1%	70
KAS	121	1	VH1-12-1	7	7,1%	79
YES8c	122	1	VH1-12-1	8	8,2%	34
RF-TS1	123	1	VH1-12-1	8	8,2%	82
BOR'	121	1	VH1-12-8	7	7,1%	79
VHGL 1.9	101	1	VH1-12-1	8	8,2%	26
mAb410.30F305	117	1	VH1-12-9	5	5,1%	52
EV1-15	127	1	VH1-12-8	10	10,2%	78
mAb112	122	1	VH1-12-1	11	11,2%	71
EU	117	1	VH1-12-1	11	11,2%	28
H210	127	1	VH1-12-1	12	12,2%	66
TRANSGENE	104	1	VH1-12-1	0	0,0%	111
CLL2-1	93	1	VH1-12-1	0	0,0%	30
CLL10 13-3	97	1	VH1-12-1	0	0,0%	29
LS7	99	1	VH1-12-7	4	4,1%	113
ALL7-1	87	1	VH1-12-7	0	0,0%	30
CLL3-1	91	1	VH1-12-7	1	1,0%	30
ALL56-1	85	1	VH1-13-8	0	0,0%	30
ALL1-1	87	1	VH1-13-6	1	1,0%	30
ALL4-1	94	1	VH1-13-8	0	0,0%	30

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
ALL56 15-4	85	1	VH1-13-8	5	5,1%	29
CLL4-1	88	1	VH1-13-1	1	1,0%	30
Au92.1	98	1	VH1-12-5	0	0,0%	49
RF-TS3	120	1	VH1-12-5	1	1,0%	82
Au4.1	98	1	VH1-12-5	1	1,0%	49
HP1	121	1	VH1-13-6	13	13,3%	110
BLI	127	1	VH1-13-15	5	5,1%	72
No.13	127	1	VH1-12-2	19	19,4%	76
TR1.23	122	1	VH1-13-2	23	23,5%	88
S1-1	125	1	VH1-12-2	18	18,4%	76
TR1.10	119	1	VH1-13-12	14	14,3%	88
E55 1.A2	102	1	VH1-13-15	3	3,1%	26
SP2	119	1	VH1-13-6	15	15,3%	89
TNF-H9G1	111	1	VH1-13-18	2	2,0%	42
G3D10H	127	1	VH1-13-16	19	19,4%	127
TR1.9	118	1	VH1-13-12	14	14,3%	88
TR1.8	121	1	VH1-12-1	24	24,5%	88
LUNm01	127	1	VH1-13-6	22	22,4%	9
K1B12H	127	1	VH1-12-7	23	23,5%	127
L3B2	99	1	VH1-13-6	2	2,0%	46
ss2	100	1	VH1-13-6	2	2,0%	46
No.86	124	1	VH1-12-1	20	20,4%	76
TR1.6	124	1	VH1-12-1	19	19,4%	88
ss7	99	1	VH1-12-7	3	3,1%	46
s5B7	102	1	VH1-12-1	0	0,0%	46
s6A3	97	1	VH1-12-1	0	0,0%	46
ss6	99	1	VH1-12-1	0	0,0%	46
L2H7	103	1	VH1-13-12	0	0,0%	46
s6BG8	93	1	VH1-13-12	0	0,0%	46
s6C9	107	1	VH1-13-12	0	0,0%	46
HIV-b4	124	1	VH1-13-12	21	21,4%	12
HIV-b12	124	1	VH1-13-12	21	21,4%	12
L3G5	98	1	VH1-13-6	1	1,0%	46
22	115	1	VH1-13-6	11	11,2%	118
L2A12	99	1	VH1-13-15	3	3,1%	46
PHOX15	124	1	VH1-12-7	20	20,4%	73

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
LUNm03	127	1	VH1-1X-1	18	18,4%	9
CEA4-8A	129	1	VH1-12-7	1	1,0%	42
M60	121	2	VH2-31-3	3	3,0%	103
HiH10	127	2	VH2-31-5	9	9,0%	4
COR	119	2	VH2-31-2	11	11,0%	91
2-115-19	124	2	VH2-31-11	8	8,1%	124
OU	125	2	VH2-31-14	20	25,6%	92
HE	120	2	VH2-31-13	19	19,0%	27
CLL33 40-1	78	2	VH2-31-5	2	2,0%	29
E55 3.9	88	3	VH3-11-5	7	7,2%	26
MTFC3	125	3	VH3-14-4	21	21,0%	131
MTFC11	125	3	VH3-14-4	21	21,0%	131
MTFJ1	114	3	VH3-14-4	21	21,0%	131
MTFJ2	114	3	VH3-14-4	21	21,0%	131
MTFUJ4	100	3	VH3-14-4	21	21,0%	131
MTFUJ5	100	3	VH3-14-4	21	21,0%	131
MTFUJ2	100	3	VH3-14-4	22	22,0%	131
MTFC8	125	3	VH3-14-4	23	23,0%	131
TD e Vq	113	3	VH3-14-4	0	0,0%	16
rMTF	114	3	VH3-14-4	5	5,0%	131
MTFUJ6	100	3	VH3-14-4	10	10,0%	131
RF-KES	107	3	VH3-14-4	9	9,0%	85
N51P8	126	3	VH3-14-1	9	9,0%	77
TEI	119	3	VH3-13-8	21	21,4%	20
33.H11	115	3	VH3-13-19	10	10,2%	129
SB1/D8	101	3	VH3-1X-8	14	14,0%	2
38P1	119	3	VH3-11-3	0	0,0%	104
BRO'IGM	119	3	VH3-11-3	13	13,4%	19
NIE	119	3	VH3-13-7	15	15,3%	87
3D6	126	3	VH3-13-26	5	5,1%	35
ZM1-1	112	3	VH3-11-3	8	8,2%	5
E55 3.15	110	3	VH3-13-26	0	0,0%	26
gF9	108	3	VH3-13-8	15	15,3%	75
THY-32	120	3	VH3-13-26	3	3,1%	42
RF-KL5	100	3	VH3-13-26	5	5,1%	96
OST577	122	3	VH3-13-13	6	6,1%	5

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>5</sup>	Reference <sup>7</sup>
BO	113	3	VH3-13-19	15	15,3%	10
TT125	121	3	VH3-13-10	15	15,3%	64
2-115-58	127	3	VH3-13-10	11	11,2%	124
KOL	126	3	VH3-13-14	16	16,3%	102
mAb60	118	3	VH3-13-17	14	14,3%	45
RF-AN	106	3	VH3-13-26	8	8,2%	85
BUT	115	3	VH3-11-6	13	13,4%	119
KOL-based CAMPATH-9	118	3	VH3-13-13	16	16,3%	41
B1	119	3	VH3-13-19	13	13,3%	53
N98P1	127	3	VH3-13-1	13	13,3%	77
TT117	107	3	VH3-13-10	12	12,2%	64
WEA	114	3	VH3-13-12	15	15,3%	40
HIL	120	3	VH3-13-14	14	14,3%	23
s5A10	97	3	VH3-13-14	0	0,0%	46
s5D11	98	3	VH3-13-7	0	0,0%	46
s6C8	100	3	VH3-13-7	0	0,0%	46
s6H12	98	3	VH3-13-7	0	0,0%	46
VH10.7	119	3	VH3-13-14	16	16,3%	128
HIV-loop2	126	3	VH3-13-7	16	16,3%	12
HIV-loop35	126	3	VH3-13-7	16	16,3%	12
TRO	122	3	VH3-13-1	13	13,3%	61
SA-4B	123	3	VH3-13-1	15	15,3%	125
L2B5	98	3	VH3-13-13	0	0,0%	46
s6E11	95	3	VH3-13-13	0	0,0%	46
s6H7	100	3	VH3-13-13	0	0,0%	46
ss1	102	3	VH3-13-13	0	0,0%	46
ss8	94	3	VH3-13-13	0	0,0%	46
DOB	120	3	VH3-13-26	21	21,4%	116
THY-33	115	3	VH3-13-15	20	20,4%	42
NOV	118	3	VH3-13-19	14	14,3%	38
rsv13H	120	3	VH3-13-24	20	20,4%	11
L3G11	98	3	VH3-13-20	2	2,0%	46
L2E8	99	3	VH3-13-19	0	0,0%	46
L2D10	101	3	VH3-13-10	1	1,0%	46
L2E7	98	3	VH3-13-10	1	1,0%	46

Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
L3A10	100	3	VH3-13-24	0	0,0%	46
L2E5	97	3	VH3-13-2	1	1,0%	46
BUR	119	3	VH3-13-7	21	21,4%	67
s4D5	107	3	VH3-11-3	1	1,0%	46
19	116	3	VH3-13-16	4	4,1%	118
s5D4	99	3	VH3-13-1	0	0,0%	46
s6A8	100	3	VH3-13-1	0	0,0%	46
HIV-loop13	123	3	VH3-13-12	17	17,3%	12
TR1.32	112	3	VH3-11-8	18	18,6%	88
L2B10	97	3	VH3-11-3	1	1,0%	46
TR1.5	114	3	VH3-11-8	21	21,6%	88
s6H9	101	3	VH3-13-25	0	0,0%	46
8	112	3	VH3-13-1	6	6,1%	118
23	115	3	VH3-13-1	6	6,1%	118
7	115	3	VH3-13-1	4	4,1%	118
TR1.3	120	3	VH3-11-8	20	20,6%	88
18/2	125	3	VH3-13-10	0	0,0%	32
18/9	125	3	VH3-13-10	0	0,0%	31
30P1	119	3	VH3-13-10	0	0,0%	106
HF2-1/17	125	3	VH3-13-10	0	0,0%	8
A77	109	3	VH3-13-10	0	0,0%	44
B19.7	108	3	VH3-13-10	0	0,0%	44
M43	119	3	VH3-13-10	0	0,0%	103
1/17	125	3	VH3-13-10	0	0,0%	31
18/17	125	3	VH3-13-10	0	0,0%	31
E54 3.4	109	3	VH3-13-10	0	0,0%	26
LAMBDA-VH26	98	3	VH3-13-10	1	1,0%	95
E54 3.8	111	3	VH3-13-10	1	1,0%	26
GL16	106	3	VH3-13-10	1	1,0%	44
4G12	125	3	VH3-13-10	1	1,0%	56
A73	106	3	VH3-13-10	2	2,0%	44
AL1.3	111	3	VH3-13-10	3	3,1%	117
3.A290	118	3	VH3-13-10	2	2,0%	108
Ab18	127	3	VH3-13-8	2	2,0%	100
E54 3.3	105	3	VH3-13-10	3	3,1%	26
35G6	121	3	VH3-13-10	3	3,1%	57



Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
A95	107	3	VH3-13-10	5	5,1%	44
Ab25	128	3	VH3-13-10	5	5,1%	100
N87	126	3	VH3-13-10	4	4,1%	77
ED8.4	99	3	VH3-13-10	6	6,1%	2
RF-KL1	122	3	VH3-13-10	6	6,1%	82
AL1.1	112	3	VH3-13-10	2	2,0%	117
AL3.11	102	3	VH3-13-10	1	1,0%	117
32.B9	127	3	VH3-13-8	6	6,1%	129
TK1	109	3	VH3-13-10	2	2,0%	117
POP	123	3	VH3-13-10	8	8,2%	115
9F2H	127	3	VH3-13-10	9	9,2%	127
VD	115	3	VH3-13-10	9	9,2%	10
Vh38Cl.10	121	3	VH3-13-10	8	8,2%	74
Vh38Cl.9	121	3	VH3-13-10	8	8,2%	74
Vh38Cl.8	121	3	VH3-13-10	8	8,2%	74
63P1	120	3	VH3-11-8	0	0,0%	104
60P2	117	3	VH3-11-8	0	0,0%	104
AL3.5	90	3	VH3-13-10	2	2,0%	117
GF4/1.1	123	3	VH3-13-10	10	10,2%	39
Ab21	126	3	VH3-13-10	12	12,2%	100
TD d Vp	118	3	VH3-13-17	2	2,0%	16
Vh38Cl.4	119	3	VH3-13-10	8	8,2%	74
Vh38Cl.5	119	3	VH3-13-10	8	8,2%	74
AL3.4	104	3	VH3-13-10	1	1,0%	117
FOG1-A3	115	3	VH3-13-19	2	2,0%	42
HA3D1	117	3	VH3-13-21	1	1,0%	81
E54 3.2	112	3	VH3-13-24	0	0,0%	26
mAb52	128	3	VH3-13-12	2	2,0%	51
mAb53	128	3	VH3-13-12	2	2,0%	51
mAb56	128	3	VH3-13-12	2	2,0%	51
mAb57	128	3	VH3-13-12	2	2,0%	51
mAb58	128	3	VH3-13-12	2	2,0%	51
mAb59	128	3	VH3-13-12	2	2,0%	51
mAb105	128	3	VH3-13-12	2	2,0%	51
mAb107	128	3	VH3-13-12	2	2,0%	51
E55 3.14	110	3	VH3-13-19	0	0,0%	26

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
F13-28	106	3	VH3-13-19	1	1,0%	94
mAb55	127	3	VH3-13-18	4	4,1%	51
YSE	117	3	VH3-13-24	6	6,1%	72
E55 3.23	106	3	VH3-13-19	2	2,0%	26
RF-TS5	101	3	VH3-13-1	3	3,1%	85
N42P5	124	3	VH3-13-2	7	7,1%	77
FOG1-H6	110	3	VH3-13-16	7	7,1%	42
O-81	115	3	VH3-13-19	11	11,2%	47
HIV-s8	122	3	VH3-13-12	11	11,2%	12
mAb114	125	3	VH3-13-19	12	12,2%	71
33.F12	116	3	VH3-13-2	4	4,1%	129
484	119	3	VH3-1X-3	0	0,0%	101
M26	123	3	VH3-1X-3	0	0,0%	103
VHGL 3.1	100	3	VH3-1X-3	0	0,0%	26
E55 3.13	113	3	VH3-1X-3	1	1,0%	26
SB5/D6	101	3	VH3-1X-6	3	3,0%	2
RAY4	101	3	VH3-1X-6	3	3,0%	2
82-D V-D	106	3	VH3-1X-3	5	5,0%	112
MAL	129	3	VH3-1X-3	5	5,0%	72
LOC	123	3	VH3-1X-6	5	5,0%	72
LSF2	101	3	VH3-1X-6	11	11,0%	2
HIB RC3	100	3	VH3-1X-6	11	11,0%	1
56P1	119	3	VH3-13-7	0	0,0%	104
M72	122	3	VH3-13-7	0	0,0%	103
M74	121	3	VH3-13-7	0	0,0%	103
E54 3.5	105	3	VH3-13-7	0	0,0%	26
2E7	123	3	VH3-13-7	0	0,0%	63
2P1	117	3	VH3-13-7	0	0,0%	104
RF-SJ2	127	3	VH3-13-7	1	1,0%	83
PR-TS1	114	3	VH3-13-7	1	1,0%	85
KIM46H	127	3	VH3-13-13	0	0,0%	18
E55 3.6	108	3	VH3-13-7	2	2,0%	26
E55 3.10	107	3	VH3-13-13	1	1,0%	26
3.B6	114	3	VH3-13-13	1	1,0%	108
E54 3.6	110	3	VH3-13-13	1	1,0%	26
FL2-2	114	3	VH3-13-13	1	1,0%	80

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
RF-SJ3	112	3	VH3-13-7	2	2,0%	85
E55 3.5	105	3	VH3-13-14	1	1,0%	26
BSA3	121	3	VH3-13-13	1	1,0%	73
HMST-1	119	3	VH3-13-7	3	3,1%	130
RF-TS2	126	3	VH3-13-13	4	4,1%	82
E55 3.12	109	3	VH3-13-15	0	0,0%	26
19.E7	126	3	VH3-13-14	3	3,1%	129
11-50	119	3	VH3-13-13	6	6,1%	130
E29.1	120	3	VH3-13-15	2	2,0%	25
E55 3.16	108	3	VH3-13-7	6	6,1%	26
TNF-E1	117	3	VH3-13-7	7	7,1%	42
RF-SJ1	127	3	VH3-13-13	6	6,1%	83
FOG1-A4	116	3	VH3-13-7	8	8,2%	42
TNF-A1	117	3	VH3-13-15	4	4,1%	42
PR-SJ2	107	3	VH3-13-14	8	8,2%	85
HN.14	124	3	VH3-13-13	10	10,2%	33
CAM'	121	3	VH3-13-7	12	12,2%	65
HIV-B8	125	3	VH3-13-7	9	9,2%	12
HIV-b27	125	3	VH3-13-7	9	9,2%	12
HIV-b8	125	3	VH3-13-7	9	9,2%	12
HIV-s4	125	3	VH3-13-7	9	9,2%	12
HIV-B26	125	3	VH3-13-7	9	9,2%	12
HIV-B35	125	3	VH3-13-7	10	10,2%	12
HIV-b18	125	3	VH3-13-7	10	10,2%	12
HIV-b22	125	3	VH3-13-7	11	11,2%	12
HIV-b13	125	3	VH3-13-7	12	12,2%	12
333	117	3	VH3-14-4	24	24,0%	24
1H1	120	3	VH3-14-4	24	24,0%	24
1B11	120	3	VH3-14-4	23	23,0%	24
CLL30 2-3	86	3	VH3-13-19	1	1,0%	29
GA	110	3	VH3-13-7	19	19,4%	36
JeB	99	3	VH3-13-14	3	3,1%	7
GAL	110	3	VH3-13-19	10	10,2%	126
K6H6	119	3	VH3-1X-6	18	18,0%	60
K4B8	119	3	VH3-1X-6	18	18,0%	60
K5B8	119	3	VH3-1X-6	18	18,0%	60

Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
K5C7	119	3	VH3-1X-6	19	19,0%	60
K5G5	119	3	VH3-1X-6	19	19,0%	60
K6F5	119	3	VH3-1X-6	19	19,0%	60
AL3.16	98	3	VH3-13-10	1	1,0%	117
N86P2	98	3	VH3-13-10	3	3,1%	77
N54P6	95	3	VH3-13-16	7	7,1%	77
LAMBDA HT112-1	126	4	VH4-11-2	0	0,0%	3
HY18	121	4	VH4-11-2	0	0,0%	43
mAb63	126	4	VH4-11-2	0	0,0%	45
FS-3	105	4	VH4-11-2	0	0,0%	86
FS-5	111	4	VH4-11-2	0	0,0%	86
FS-7	107	4	VH4-11-2	0	0,0%	86
FS-8	110	4	VH4-11-2	0	0,0%	86
PR-TS2	105	4	VH4-11-2	0	0,0%	85
RF-TMC	102	4	VH4-11-2	0	0,0%	85
mAb216	122	4	VH4-11-2	1	1,0%	15
mAb410.7.F91	122	4	VH4-11-2	1	1,0%	52
mAbA6H4C5	124	4	VH4-11-2	1	1,0%	15
Ab44	127	4	VH4-11-2	2	2,1%	100
6H-3C4	124	4	VH4-11-2	3	3,1%	59
FS-6	108	4	VH4-11-2	6	6,2%	86
FS-2	114	4	VH4-11-2	6	6,2%	84
HIG1	126	4	VH4-11-2	7	7,2%	62
FS-4	105	4	VH4-11-2	8	8,2%	86
SA-4A	123	4	VH4-11-2	9	9,3%	125
LES-C	119	4	VH4-11-2	10	10,3%	99
DI	78	4	VH4-11-9	16	16,5%	58
Ab26	126	4	VH4-31-4	8	8,1%	100
TS2	124	4	VH4-31-12	15	15,2%	110
265-695	115	4	VH4-11-7	16	16,5%	5
WAH	129	4	VH4-31-13	19	19,2%	93
268-D	122	4	VH4-11-8	22	22,7%	6
58P2	118	4	VH4-11-8	0	0,0%	104
mAb67	128	4	VH4-21-4	1	1,0%	45
4.L39	115	4	VH4-11-8	2	2,1%	108
mF7	111	4	VH4-31-13	3	3,0%	75

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
33.C9	122	4	VH4-21-5	7	7,1%	129
Pag-1	124	4	VH4-11-16	5	5,2%	50
B3	123	4	VH4-21-3	8	8,2%	53
IC4	120	4	VH4-11-8	6	6,2%	70
C6B2	127	4	VH4-31-12	4	4,0%	48
N78	118	4	VH4-11-9	11	11,3%	77
B2	109	4	VH4-11-8	12	12,4%	53
WRD2	123	4	VH4-11-12	6	6,2%	90
mAb426.4.2F20	126	4	VH4-11-8	2	2,1%	52
E54 4.58	115	4	VH4-11-8	1	1,0%	26
WRD6	123	4	VH4-11-12	10	10,3%	90
mAb426.12.3F1.4	122	4	VH4-11-9	4	4,1%	52
E54 4.2	108	4	VH4-21-6	2	2,0%	26
WIL	127	4	VH4-31-13	0	0,0%	90
COF	126	4	VH4-31-13	0	0,0%	90
LAR	122	4	VH4-31-13	2	2,0%	90
WAT	125	4	VH4-31-13	4	4,0%	90
mAb61	123	4	VH4-31-13	5	5,1%	45
WAG	127	4	VH4-31-4	0	0,0%	90
RF-SJ4	108	4	VH4-31-12	2	2,0%	85
E54 4.4	110	4	VH4-11-7	0	0,0%	26
E55 4.A1	108	4	VH4-11-7	0	0,0%	26
PR-SJ1	103	4	VH4-11-7	1	1,0%	85
E54 4.23	111	4	VH4-11-7	1	1,0%	26
CLL7 7-2	97	4	VH4-11-12	0	0,0%	29
37P1	95	4	VH4-11-12	0	0,0%	104
ALL52 30-2	91	4	VH4-31-12	4	4,0%	29
EBV-21	98	5	VH5-12-1	0	0,0%	13
CB-4	98	5	VH5-12-1	0	0,0%	13
CLL-12	98	5	VH5-12-1	0	0,0%	13
L3-4	98	5	VH5-12-1	0	0,0%	13
CLL11	98	5	VH5-12-1	0	0,0%	17
CORD3	98	5	VH5-12-1	0	0,0%	17
CORD4	98	5	VH5-12-1	0	0,0%	17
CORD8	98	5	VH5-12-1	0	0,0%	17
CORD9	98	5	VH5-12-1	0	0,0%	17

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Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
CD+1	98	5	VH5-12-1	0	0,0%	17
CD+3	98	5	VH5-12-1	0	0,0%	17
CD+4	98	5	VH5-12-1	0	0,0%	17
CD-1	98	5	VH5-12-1	0	0,0%	17
CD-5	98	5	VH5-12-1	0	0,0%	17
VERG14	98	5	VH5-12-1	0	0,0%	17
PBL1	98	5	VH5-12-1	0	0,0%	17
PBL10	98	5	VH5-12-1	0	0,0%	17
STRAb SA-1A	127	5	VH5-12-1	0	0,0%	125
DOB'	122	5	VH5-12-1	0	0,0%	97
VERG5	98	5	VH5-12-1	0	0,0%	17
PBL2	98	5	VH5-12-1	1	1,0%	17
Tu16	119	5	VH5-12-1	1	1,0%	49
PBL12	98	5	VH5-12-1	1	1,0%	17
CD+2	98	5	VH5-12-1	1	1,0%	17
CORD10	98	5	VH5-12-1	1	1,0%	17
PBL9	98	5	VH5-12-1	1	1,0%	17
CORD2	98	5	VH5-12-1	2	2,0%	17
PBL6	98	5	VH5-12-1	2	2,0%	17
CORD5	98	5	VH5-12-1	2	2,0%	17
CD-2	98	5	VH5-12-1	2	2,0%	17
CORD1	98	5	VH5-12-1	2	2,0%	17
CD-3	98	5	VH5-12-1	3	3,1%	17
VERG4	98	5	VH5-12-1	3	3,1%	17
PBL13	98	5	VH5-12-1	3	3,1%	17
PBL7	98	5	VH5-12-1	3	3,1%	17
HAN	119	5	VH5-12-1	3	3,1%	97
VERG3	98	5	VH5-12-1	3	3,1%	17
PBL3	98	5	VH5-12-1	3	3,1%	17
VERG7	98	5	VH5-12-1	3	3,1%	17
PBL5	94	5	VH5-12-1	0	0,0%	17
CD-4	98	5	VH5-12-1	4	4,1%	17
CLL10	98	5	VH5-12-1	4	4,1%	17
PBL11	98	5	VH5-12-1	4	4,1%	17
CORD6	98	5	VH5-12-1	4	4,1%	17
VERG2	98	5	VH5-12-1	5	5,1%	17

Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
83P2	119	5	VH5-12-1	0	0,0%	103
VERG9	98	5	VH5-12-1	6	6,1%	17
CLL6	98	5	VH5-12-1	6	6,1%	17
PBL8	98	5	VH5-12-1	7	7,1%	17
Ab2022	120	5	VH5-12-1	3	3,1%	100
CAV	127	5	VH5-12-4	0	0,0%	97
HOW	120	5	VH5-12-4	0	0,0%	97
PET	127	5	VH5-12-4	0	0,0%	97
ANG	121	5	VH5-12-4	0	0,0%	97
KER	121	5	VH5-12-4	0	0,0%	97
5.M13	118	5	VH5-12-4	0	0,0%	107
Au2.1	118	5	VH5-12-4	1	1,0%	49
WS1	126	5	VH5-12-1	9	9,2%	110
TD Vn	98	5	VH5-12-4	1	1,0%	16
TEL13	116	5	VH5-12-1	9	9,2%	73
E55 5.237	112	5	VH5-12-4	2	2,0%	26
VERG1	98	5	VH5-12-1	10	10,2%	17
CD4-74	117	5	VH5-12-1	10	10,2%	42
257-D	125	5	VH5-12-1	11	11,2%	6
CLL4	98	5	VH5-12-1	11	11,2%	17
CLL8	98	5	VH5-12-1	11	11,2%	17
Ab2	124	5	VH5-12-1	12	12,2%	120
Vh383ex	98	5	VH5-12-1	12	12,2%	120
CLL3	98	5	VH5-12-2	11	11,2%	17
Au59.1	122	5	VH5-12-1	12	12,2%	49
TEL16	117	5	VH5-12-1	12	12,2%	73
M61	104	5	VH5-12-1	0	0,0%	103
Tu0	99	5	VH5-12-1	5	5,1%	49
P2-51	122	5	VH5-12-1	13	13,3%	121
P2-54	122	5	VH5-12-1	11	11,2%	121
P1-56	119	5	VH5-12-1	9	9,2%	121
P2-53	122	5	VH5-12-1	10	10,2%	121
P1-51	123	5	VH5-12-1	19	19,4%	121
P1-54	123	5	VH5-12-1	3	3,1%	121
P3-69	127	5	VH5-12-1	4	4,1%	121
P3-9	119	5	VH5-12-1	4	4,1%	121

Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
1-185-37	125	5	VH5-12-4	0	0,0%	124
1-187-29	125	5	VH5-12-4	0	0,0%	124
P1-58	128	5	VH5-12-4	10	10,2%	121
P2-57	118	5	VH5-12-4	3	3,1%	121
P2-55	123	5	VH5-12-1	5	5,1%	121
P2-56	123	5	VH5-12-1	20	20,4%	121
P2-52	122	5	VH5-12-1	11	11,2%	121
P3-60	122	5	VH5-12-1	8	8,2%	121
P1-57	123	5	VH5-12-1	4	4,1%	121
P1-55	122	5	VH5-12-1	14	14,3%	121
MD3-4	128	5	VH5-12-4	12	12,2%	5
P1-52	121	5	VH5-12-1	11	11,2%	121
CLL5	98	5	VH5-12-1	13	13,3%	17
CLL7	98	5	VH5-12-1	14	14,3%	17
L2F10	100	5	VH5-12-1	1	1,0%	46
L3B6	98	5	VH5-12-1	1	1,0%	46
VH6.A12	119	6	VH6-35-1	13	12,9%	122
s5A9	102	6	VH6-35-1	1	1,0%	46
s6G4	99	6	VH6-35-1	1	1,0%	46
ss3	99	6	VH6-35-1	1	1,0%	46
6-1G1	101	6	VH6-35-1	0	0,0%	14
F19L16	107	6	VH6-35-1	0	0,0%	68
L16	120	6	VH6-35-1	0	0,0%	69
M71	121	6	VH6-35-1	0	0,0%	103
ML1	120	6	VH6-35-1	0	0,0%	69
F19ML1	107	6	VH6-35-1	0	0,0%	68
15P1	127	6	VH6-35-1	0	0,0%	104
VH6.N1	121	6	VH6-35-1	0	0,0%	122
VH6.N11	123	6	VH6-35-1	0	0,0%	122
VH6.N12	123	6	VH6-35-1	0	0,0%	122
VH6.N2	125	6	VH6-35-1	0	0,0%	122
VH6.N5	125	6	VH6-35-1	0	0,0%	122
VH6.N6	127	6	VH6-35-1	0	0,0%	122
VH6.N7	126	6	VH6-35-1	0	0,0%	122
VH6.N8	123	6	VH6-35-1	0	0,0%	122
VH6.N9	123	6	VH6-35-1	0	0,0%	122



Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
VH6.N10	123	6	VH6-35-1	0	0,0%	122
VH6.A3	123	6	VH6-35-1	0	0,0%	122
VH6.A1	124	6	VH6-35-1	0	0,0%	122
VH6.A4	120	6	VH6-35-1	0	0,0%	122
E55 6.16	116	6	VH6-35-1	0	0,0%	26
E55 6.17	120	6	VH6-35-1	0	0,0%	26
E55 6.6	120	6	VH6-35-1	0	0,0%	26
VHGL 6.3	102	6	VH6-35-1	0	0,0%	26
CB-201	118	6	VH6-35-1	0	0,0%	109
VH6.N4	122	6	VH6-35-1	0	0,0%	122
E54 6.4	109	6	VH6-35-1	1	1,0%	26
VH6.A6	126	6	VH6-35-1	1	1,0%	122
E55 6.14	120	6	VH6-35-1	1	1,0%	26
E54 6.6	107	6	VH6-35-1	1	1,0%	26
E55 6.10	112	6	VH6-35-1	1	1,0%	26
E54 6.1	107	6	VH6-35-1	2	2,0%	26
E55 6.13	120	6	VH6-35-1	2	2,0%	26
E55 6.3	120	6	VH6-35-1	2	2,0%	26
E55 6.7	116	6	VH6-35-1	2	2,0%	26
E55 6.2	120	6	VH6-35-1	2	2,0%	26
E55 6.X	111	6	VH6-35-1	2	2,0%	26
E55 6.11	111	6	VH6-35-1	3	3,0%	26
VH6.A11	118	6	VH6-35-1	3	3,0%	122
A10	107	6	VH6-35-1	3	3,0%	68
E55 6.1	120	6	VH6-35-1	4	4,0%	26
FK-001	124	6	VH6-35-1	4	4,0%	65
VH6.A5	121	6	VH6-35-1	4	4,0%	122
VH6.A7	123	6	VH6-35-1	4	4,0%	122
HBp2	119	6	VH6-35-1	4	4,0%	4
Au46.2	123	6	VH6-35-1	5	5,0%	49
A431	106	6	VH6-35-1	5	5,0%	68
VH6.A2	120	6	VH6-35-1	5	5,0%	122
VH6.A9	125	6	VH6-35-1	8	7,9%	122
VH6.A8	118	6	VH6-35-1	10	9,9%	122
VH6-FF3	118	6	VH6-35-1	2	2,0%	123
VH6.A10	126	6	VH6-35-1	12	11,9%	122

Table 2C: (continued)

Name <sup>1</sup>	aa <sup>2</sup>	Computed family <sup>3</sup>	Germline gene <sup>4</sup>	Diff. to germline <sup>5</sup>	% diff. to germline <sup>6</sup>	Reference <sup>7</sup>
VH6-EB10	117	6	VH6-35-1	3	3,0%	123
VH6-E6	119	6	VH6-35-1	6	5,9%	123
VH6-FE2	121	6	VH6-35-1	6	5,9%	123
VH6-EE6	116	6	VH6-35-1	6	5,9%	123
VH6-FD10	118	6	VH6-35-1	6	5,9%	123
VH6-EX8	113	6	VH6-35-1	6	5,9%	123
VH6-FG9	121	6	VH6-35-1	8	7,9%	123
VH6-E5	116	6	VH6-35-1	9	8,9%	123
VH6-EC8	122	6	VH6-35-1	9	8,9%	123
VH6-E10	120	6	VH6-35-1	10	9,9%	123
VH6-FF11	122	6	VH6-35-1	11	10,9%	123
VH6-FD2	115	6	VH6-35-1	11	10,9%	123
CLL10 17-2	88	6	VH6-35-1	4	4,0%	29
VH6-BB11	94	6	VH6-35-1	4	4,0%	123
VH6-B4I	93	6	VH6-35-1	7	6,9%	123
JU17	102	6	VH6-35-1	3	3,0%	114
VH6-BD9	96	6	VH6-35-1	11	10,9%	123
VH6-BB9	94	6	VH6-35-1	12	11,9%	123

Table 3A: assignment of rearranged V kappa sequences to their germline counterparts

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
1	Vk1-1	28	
1	Vk1-2	0	
1	Vk1-3	1	
1	Vk1-4	0	
1	Vk1-5	7	
1	Vk1-6	0	
1	Vk1-7	0	
1	Vk1-8	2	
1	Vk1-9	9	
1	Vk1-10	0	
1	Vk1-11	1	
1	Vk1-12	7	
1	Vk1-13	1	
1	Vk1-14	7	
1	Vk1-15	2	
1	Vk1-16	2	
1	Vk1-17	16	
1	Vk1-18	1	
1	Vk1-19	33	
1	Vk1-20	1	
1	Vk1-21	1	
1	Vk1-22	0	
1	Vk1-23	0	<i>119 entries</i>
2	Vk2-1	0	
2	Vk2-2	1	
2	Vk2-3	0	
2	Vk2-4	0	
2	Vk2-5	0	
2	Vk2-6	16	
2	Vk2-7	0	
2	Vk2-8	0	
2	Vk2-9	1	
2	Vk2-10	0	
2	Vk2-11	7	
2	Vk2-12	0	<i>25 entries</i>
3	Vk3-1	1	
3	Vk3-2	0	

Table 3A: (continued)

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
3	Vk3-3	35	
3	Vk3-4	115	
3	Vk3-5	0	
3	Vk3-6	0	
3	Vk3-7	1	
3	Vk3-8	40	<i>192 entries</i>
4	Vk4-1	33	<i>33 entries</i>
5	Vk5-1	1	<i>1 entry</i>
6	Vk6-1	0	
6	Vk6-2	0	<i>0 entries</i>
7	Vk7-1	0	<i>0 entries</i>

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Table 3B: assignment of rearranged V lambda sequences to their germline counterparts

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
1	DPL1	1	
1	DPL2	14	
1	DPL3	6	
1	DPL4	1	
1	HUMLV117	4	
1	DPL5	13	
1	DPL6	0	
1	DPL7	0	
1	DPL8	3	
1	DPL9	0	42 entries
2	DPL10	5	
2	VLAMBDA 2.1	0	
2	DPL11	23	
2	DPL12	15	
2	DPL13	0	
2	DPL14	0	43 entries
3	DPL16	10	
3	DPL23	19	
3	Humlv318	9	38 entries
7	DPL18	1	
7	DPL19	0	1 entries
8	DPL21	2	
8	HUMLV801	6	8 entries
9	DPL22	0	0 entries
unassigned	DPL24	0	0 entries
10	gVLX-4.4	0	0 entries

Table 3C: assignment of rearranged V heavy chain sequences to their germline counterparts

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
1	VH1-12-1	38	
1	VH1-12-8	2	
1	VH1-12-2	2	
1	VH1-12-9	2	
1	VH1-12-3	0	
1	VH1-12-4	0	
1	VH1-12-5	3	
1	VH1-12-6	0	
1	VH1-12-7	23	
1	VH1-13-1	1	
1	VH1-13-2	1	
1	VH1-13-3	0	
1	VH1-13-4	0	
1	VH1-13-5	0	
1	VH1-13-6	17	
1	VH1-13-7	0	
1	VH1-13-8	3	
1	VH1-13-9	0	
1	VH1-13-10	0	
1	VH1-13-11	0	
1	VH1-13-12	10	
1	VH1-13-13	0	
1	VH1-13-14	0	
1	VH1-13-15	4	
1	VH1-13-16	2	
1	VH1-13-17	0	
1	VH1-13-18	1	
1	VH1-13-19	0	
1	VH1-1X-1	1	110 entries
2	VH2-21-1	0	
2	VH2-31-1	0	
2	VH2-31-2	1	
2	VH2-31-3	1	
2	VH2-31-4	0	
2	VH2-31-5	2	
2	VH2-31-6	0	
2	VH2-31-7	0	

Table 3C: (continued)

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
2	VH2-31-14	1	
2	VH2-31-8	0	
2	VH2-31-9	0	
2	VH2-31-10	0	
2	VH2-31-11	1	
2	VH2-31-12	0	
2	VH2-31-13	1	7 entries
3	VH3-11-1	0	
3	VH3-11-2	0	
3	VH3-11-3	5	
3	VH3-11-4	0	
3	VH3-11-5	1	
3	VH3-11-6	1	
3	VH3-11-7	0	
3	VH3-11-8	5	
3	VH3-13-1	9	
3	VH3-13-2	3	
3	VH3-13-3	0	
3	VH3-13-4	0	
3	VH3-13-5	0	
3	VH3-13-6	0	
3	VH3-13-7	32	
3	VH3-13-8	4	
3	VH3-13-9	0	
3	VH3-13-10	46	
3	VH3-13-11	0	
3	VH3-13-12	11	
3	VH3-13-13	17	
3	VH3-13-14	8	
3	VH3-13-15	4	
3	VH3-13-16	3	
3	VH3-13-17	2	
3	VH3-13-18	1	
3	VH3-13-19	13	
3	VH3-13-20	1	
3	VH3-13-21	1	
3	VH3-13-22	0	

Table 3C: (continued)

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
3	VH3-13-23	0	
3	VH3-13-24	4	
3	VH3-13-25	1	
3	VH3-13-26	6	
3	VH3-14-1	1	
3	VH3-14-4	15	
3	VH3-14-2	0	
3	VH3-14-3	0	
3	VH3-1X-1	0	
3	VH3-1X-2	0	
3	VH3-1X-3	6	
3	VH3-1X-4	0	
3	VH3-1X-5	0	
3	VH3-1X-6	11	
3	VH3-1X-7	0	
3	VH3-1X-8	1	
3	VH3-1X-9	0	212 entries
4	VH4-11-1	0	
4	VH4-11-2	20	
4	VH4-11-3	0	
4	VH4-11-4	0	
4	VH4-11-5	0	
4	VH4-11-6	0	
4	VH4-11-7	5	
4	VH4-11-8	7	
4	VH4-11-9	3	
4	VH4-11-10	0	
4	VH4-11-11	0	
4	VH4-11-12	4	
4	VH4-11-13	0	
4	VH4-11-14	0	
4	VH4-11-15	0	
4	VH4-11-16	1	
4	VH4-21-1	0	
4	VH4-21-2	0	
4	VH4-21-3	1	
4	VH4-21-4	1	



Table 3C: (continued)

Family <sup>1</sup>	Name	Rearranged <sup>2</sup>	Sum
4	VH4-21-5	1	
4	VH4-21-6	1	
4	VH4-21-7	0	
4	VH4-21-8	0	
4	VH4-21-9	0	
4	VH4-31-1	0	
4	VH4-31-2	0	
4	VH4-31-3	0	
4	VH4-31-4	2	
4	VH4-31-5	0	
4	VH4-31-6	0	
4	VH4-31-7	0	
4	VH4-31-8	0	
4	VH4-31-9	0	
4	VH4-31-10	0	
4	VH4-31-11	0	
4	VH4-31-12	4	
4	VH4-31-13	7	
4	VH4-31-14	0	
4	VH4-31-15	0	
4	VH4-31-16	0	
4	VH4-31-17	0	
4	VH4-31-18	0	
4	VH4-31-19	0	
4	VH4-31-20	0	57 entries
5	VH5-12-1	82	
5	VH5-12-2	1	
5	VH5-12-3	0	
5	VH5-12-4	14	97 entries
6	VH6-35-1	74	74 entries

Table 4A: Analysis of V kappa subgroup 1

Framework I																
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A		1							1				102		1	
B			1			1										
C														1		
D	64															
E	8	14													1	
F									1	6				1		
G																105
H																
I		65													4	
K			1													
L		6	21								96		1			
M	1		66													
N																
P								103		1		2			1	
Q		62			88						1					
R																
S						89		102	80		103		103			
T		1		88					18							
V		1	9								8		2		98	
W																
X	1															
Y																
-																
unknown (?)																
not sequenced <sup>2</sup>	31	31	18	18	17	16	16	2	1							
sum of seq <sup>3</sup>	74	74	87	87	88	89	89	103	104	105	105	105	105	105	105	105
oomcaa <sup>3</sup>	64	65	62	66	88	88	89	103	102	80	96	103	102	103	98	105
mcaa <sup>4</sup>	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G
rel. oomcaa <sup>5</sup>	86%	88%	71%	76%	100%	99%	100%	100%	98%	76%	91%	98%	97%	98%	93%	100%
pos occupied <sup>6</sup>	4	5	5	2	1	2	1	1	3	4	3	2	3	3	5	1

Table 4A: Analysis of V kappa subgroup 1

amino acid <sup>1</sup>	17	18	19	20	21	22	23	24	25	26	27	A	B	C	D
A			1	1		1			103						
B											1				
C							105								
D	101														
E	2							1	1		2				
F					2										
G										1					
H											1				
I			6	4	101	1									
K								2			1				
L								1							
M															
N										1					
P															
Q								20			100				
R		94						81							
S		5		1						102					
T		6		99		103			1	1					
V			98		2										
W															
X	1														
Y	1														
-												105	105	105	105
unknown (?)															
not sequenced															
sum of seq <sup>2</sup>	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
oomcaa <sup>3</sup>	101	94	98	99	101	103	105	81	103	102	100	105	105	105	105
mcaa <sup>4</sup>	D	R	V	T	I	T	C	R	A	S	Q	-	-	-	-
rel. oomcaa <sup>5</sup>	96%	90%	93%	94%	96%	98%	100%	77%	98%	97%	95%	100%	100%	100%	100%
pos occupied <sup>6</sup>	4	3	3	4	3	3	1	5	3	4	5	1	1	1	1

Table 4A: Analysis of V kappa subgroup 1

amino acid <sup>1</sup>	CDRI														
	E	F	28	29	30	31	32	33	34	35	36	37	38	39	40
A					1	1		1	42						
B												1	1		
C							1								
D			25		1	5	7					1			
E							1					2			
F				1	1		7				6				
G			25		7	3			4						
H					1	2	2		1			2			
I				98	1	4			1						
K						7								95	
L					2	1		101							
M															
N			6		16	42			50						
P															102
Q												98	103	2	
R					16	3	2							3	1
S			41	2	57	32	3	1	1						1
T			7			4			4					1	
V			1	4	1			1							
W							21			104					
X									1						
Y					1		60				98				
-	105	105													
unknown (?)														3	
not sequenced						1	1	1	1	1	1	1	1	1	1
sum of seq <sup>2</sup>	105	105	105	105	105	104	104	104	104	104	104	104	104	104	104
oomcaa <sup>3</sup>	105	105	41	98	57	42	60	101	50	104	98	98	103	95	102
mcaa <sup>4</sup>	-	-	S	I	S	N	Y	L	N	W	Y	Q	Q	K	P
rel. oomcaa <sup>5</sup>	100%	100%	39%	93%	54%	40%	58%	97%	48%	100%	94%	94%	99%	91%	98%
pos occupied <sup>6</sup>	1	1	6	4	12	11	9	4	8	1	2	5	2	4	3

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Table 4A: Analysis of V kappa subgroup 1

	Framework II									CDR II					
amino acid <sup>1</sup>	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
A			94							50	95				
B															
C															
D										21	1	1	1		
E	1	3			1	1				1		1			33
F						1			3			1			
G	100		1							9	2				
H									2						1
I		1				1	100						1		
K		95			86					16			2		5
L		1				89	103							101	
M								2							
N					10					2		1	25		
P				104						1					1
Q		1			1										62
R					3	3							1	1	2
S					1				5	1	1	99	41	2	
T		3			1					1	4	1	31		
V			9			9					1		1		
W															
X					1								1		
Y									92	1					
-															
unknown (?)	3														
not sequenced	1	1	1	1	1	1	2	3	3	2	1	1	1	1	1
sum of seq <sup>2</sup>	104	104	104	104	104	104	103	102	102	103	104	104	104	104	104
oomcaa <sup>3</sup>	100	95	94	104	86	89	103	100	92	50	95	99	41	101	62
mcaa <sup>4</sup>	G	K	A	P	K	L	L	I	Y	A	A	S	S	L	Q
rel. oomcaa <sup>5</sup>	96%	91%	90%	100%	83%	86%	100%	98%	90%	49%	91%	95%	39%	97%	60%
pos occupied <sup>6</sup>	2	6	3	1	8	6	1	2	4	10	6	6	9	3	6

Table 4A: Analysis of V kappa subgroup 1

amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
A	3										2	1	1	1	
B				1											
C															
D	1														67
E													1		30
F			1				103					3			
G	2	105							105	4	101		102		
H															3
I	3		4				1	3							
K	1					1									1
L								1							
M														1	
N	6														
P	1			101	2										
Q										1					
R	1					103		1		1	1			2	
S	68			2	103			98		96		100			
T	19			1		1		2		3				101	
V			99				1								1
W															
X			1								1		1		2
Y												1			1
-															
unknown (?)															
not sequenced															
sum of seq <sup>2</sup>	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
oomcaa <sup>3</sup>	68	105	99	101	103	103	103	98	105	96	101	100	102	101	67
mcaa <sup>4</sup>	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D
rel. oomcaa <sup>5</sup>	65%	100%	94%	96%	98%	98%	98%	93%	100%	91%	96%	95%	97%	96%	64%
pos occupied <sup>6</sup>	10	1	4	4	2	3	3	5	1	5	4	4	4	4	7

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Table 4A: Analysis of V kappa subgroup 1

Framework III																
amino acid <sup>1</sup>	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	
A		3				1				2				101	1	
B					1				3		2					
C																
D						1					16	101				
E											83					
F	102	1	21										73			
G							4				1			2		
H																
I					99	5							17			
K																
L			81					103	1				1			
M															1	
N						7	4								1	
P										97					1	
Q									97							
R						2	1		2							
S		2		1		86	94			4			1			
T		98		102		2	1								97	
V	1		2		4			1					11		1	
W																
X				1							1	2				
Y	1															
-																
unknown (?)																
not sequenced	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3
sum of seq <sup>2</sup>	104	104	104	104	104	104	104	104	103	103	103	103	103	103	102	
oomcaa <sup>3</sup>	102	98	81	102	99	86	94	103	97	97	83	101	73	101	97	
mcaa <sup>4</sup>	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	
rel. oomcaa <sup>5</sup>	98%	94%	78%	98%	95%	83%	90%	99%	94%	94%	81%	98%	71%	98%	95%	
pos occupied <sup>6</sup>	3	4	3	3	3	7	5	2	4	3	5	2	5	2	6	

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Table 4A: Analysis of V kappa subgroup 1

amino acid <sup>1</sup>	CDR III															
	86	87	88	89	90	91	92	93	94	95	A	B	C	D	E	F
A					1	7	1		5	1						
B				2	3											
C			102													
D							23	5	1							
E							1	1		1	1					
F		7				3			13							
G						1		1	2	1		1				
H		1		4	6	7	3	1								
I							4	1	2	1						
K	1				7		1									
L				7		6	2		18	2						
M																
N						6	31	19	1							
P									1	82	6					
Q				90	86	1	2									
R						1		2	2							
S	1					27	3	58	5	10						
T						3	1	15	25							
V									5							
W									1							
X																
Y	101	93				42	32	1	23							
-										3	82	88	89	89	89	89
unknown (?)		1														
not sequenced	2	3	3	2	2	1	1	1	1	4	16	16	16	16	16	16
sum of seq <sup>2</sup>	103	102	102	103	103	104	104	104	104	101	89	89	89	89	89	89
oomcaa <sup>3</sup>	101	93	102	90	86	42	32	58	25	82	82	88	89	89	89	89
mcaa <sup>4</sup>	Y	Y	C	Q	Q	Y	Y	S	T	P	-	-	-	-	-	-
rel. oomcaa <sup>5</sup>	98%	91%	100%	87%	83%	40%	31%	56%	24%	81%	92%	99%	100%	100%	100%	100%
pos occupied <sup>6</sup>	3	3	1	4	5	11	12	10	14	8	3	2	1	1	1	1

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Table 4A: Analysis of V kappa subgroup 1

amino acid <sup>1</sup>	Framework IV																	sum
	96	97	98	99	100	101	102	103	104	105	106	A	107	108				
A	1															627		
B					1					1						19		
C																209		
D	1									15						459		
E					2					65						258		
F	6		86								2					451		
G				87	29	87									2	894		
H	2	1														40		
I	5								1		72					606		
K	1	1						77						79		480		
L	18	1	1						22	4	2					793		
M		1									5					77		
N	1										1		2			232		
P	6				7										1	620		
Q	1				48					1						865		
R	6							6					2	70		413		
S	2	2														1636		
T	2	82					87	3					2			1021		
V	2							1	63		3					440		
W	15															141		
X																14		
Y	16															564		
-	4	1										85		1		1250		
unknown (?)																7		
not sequenced	16	16	18	18	18	18	18	18	19	19	20	20	20	31		589		
sum of seq <sup>2</sup>	89	89	87	87	87	87	87	87	86	86	85	85	85	74				
oomcaa <sup>3</sup>	18	82	86	87	48	87	87	77	63	65	72	85	79	70				
mcaa <sup>4</sup>	L	T	F	G	G	G	T	K	V	E	I	-	K	R				
rel. oomcaa <sup>5</sup>	20%	92%	99%	100%	55%	100%	100%	89%	73%	76%	85%	100%	93%	95%				
pos occupied <sup>6</sup>	17	7	2	1	5	1	1	4	3	5	6	1	4	4				

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Table 4B: Analysis of V kappa subgroup 2

Framework I																					
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A																			22		
B																					
C																					
D	14																				
E	3																15				
F									1	1											
G																22					
H																					
I		8																			22
K																					
L		3		1					17	18					6						
M				15																	
N																					
P								18				18			15			22			
Q						18											7				
R																					
S							18			17										22	
T					17									21							
V		6	17	1									18								
W																					
X																					
Y																					
-																					
unknown (?)					1																
not sequenced	5	5	5	5	4	4	4	4	4	4	4	4	4	4	1	1					
sum of seq <sup>2</sup>	17	17	17	17	18	18	18	18	18	18	18	18	18	21	21	22	22	22	22	22	22
oomcaa <sup>3</sup>	14	8	17	15	17	18	18	18	17	17	18	18	18	21	15	22	15	22	22	22	22
mcaa <sup>4</sup>	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A	S	I
rel. oomcaa <sup>5</sup>	82%	47%	100%	88%	94%	100%	100%	100%	94%	94%	100%	100%	100%	100%	71%	100%	68%	100%	100%	100%	100%
pos occupied <sup>6</sup>	2	3	1	3	1	1	1	1	2	2	1	1	1	1	2	1	2	1	1	1	1

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Table 4B: Analysis of V kappa subgroup 2

	CDRI																													
amino acid <sup>1</sup>	22	23	24	25	26	27	A	B	C	D	E	F	28	29	30	31	32	33	34	35	36									
A																														
B																														
C		22																												
D										1			9		1	1			11											
E																														
F															2												7			
G											1			22																
H										16								1		1										
I																														
K			1														1													
L						1		22	13										22											
M									1																					
N													10		7	12				9										
P																														
Q	1					21																								
R			21								2																			
S	21			22	22		22				19		1																	
T																8														
V									8																					
W										1																22				
X													1		1					1										
Y										4			1		11		21										15			
-												22																		
unknown (?)																														
not sequenced																														
sum of seq <sup>2</sup>	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22		
oomcaa <sup>1</sup>	21	22	21	22	22	21	22	22	13	16	19	22	10	22	11	12	21	22	11	22	15									
mcaa <sup>1</sup>	S	C	R	S	S	Q	S	L	L	H	S	-	N	G	Y	N	Y	L	D	W	Y									
rel. oomcaa <sup>3</sup>	95%	100%	95%	100%	100%	95%	100%	100%	59%	73%	86%	100%	45%	100%	50%	55%	95%	100%	50%	100%	68%									
pos occupied <sup>4</sup>	2	1	2	1	1	2	1	1	3	4	3	1	5	1	5	4	2	1	4	1	2									

Table 4B: Analysis of V kappa subgroup 2

	Framework II													CDR II												
amino acid <sup>1</sup>	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57					
A																			14							
B																										
C																										
D																			7							
E									1																	
F																										
G				22											12				1	22						
H																										
I										1	22															
K			15											5												
L	16									14	21			14	1											
M																										
N																	18									
P				22				21																		
Q	6	22				22			12					1												
R			7						8	7				1				22								
S							21								2	22	2			22						
T																	1									
V											1				6											
W																										
X																										
Y													21				1									
-																										
unknown (?)																										
not sequenced							1	1	1				1	1	1											
sum of seq <sup>1</sup>	22	22	22	22	22	22	21	21	21	22	22	22	21	21	21	22	22	22	22	22	22					
oomcaa <sup>1</sup>	16	22	15	22	22	22	21	21	12	14	21	22	21	14	12	22	18	22	14	22	22					
mcaa <sup>1</sup>	L	Q	K	P	G	Q	S	P	Q	L	L	I	Y	L	G	S	N	R	A	S	G					
rel. oomcaa <sup>1</sup>	73%	100%	68%	100%	100%	100%	100%	100%	57%	64%	95%	100%	100%	67%	57%	100%	82%	100%	64%	100%	100%					
pos occupied <sup>1</sup>	2	1	2	1	1	1	1	1	3	3	2	1	1	4	4	1	4	1	3	1	1					

Table 4B: Analysis of V kappa subgroup 2

	Framework III																				
amino acid <sup>1</sup>	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78
A																					
B																					
C																					
D			22				1				1		22								
E																					
F					21									22							
G							21		22		21										
H																					
I																	1	21			
K																	19				
L																21	1				
M																					
N																					
P		22																			
Q																					
R				20				1												20	
S				1		22		21		22									20	1	
T				1								22			21				1		
V	22				1																21
W																					
X																					
Y																					
-																					
unknown (?)															1						
not sequenced																1	1	1	1	1	1
sum of seq <sup>2</sup>	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	21	21	21	21	21	21
oomcaa <sup>3</sup>	22	22	22	20	21	22	21	21	22	22	21	22	22	22	21	21	19	21	20	20	21
mcaa <sup>4</sup>	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V
rel. oomcaa <sup>5</sup>	100%	100%	100%	91%	95%	100%	95%	95%	100%	100%	95%	100%	100%	100%	95%	100%	90%	100%	95%	95%	100%
pos occupied <sup>6</sup>	1	1	1	3	2	1	2	2	1	1	2	1	1	1	1	1	3	1	2	2	1

Table 4B: Analysis of V kappa subgroup 2

amino acid <sup>1</sup>	CDR III																A	B	C	D
	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95			
A		20											14			1				
B												1			1					
C										21										
D			1	21																
E	19		20																	
F																				
G	1					21							6			1		2		
H													1	7						
I							1									1				
K																				
L							1						12				2			
M										21										
N																				
P		1																		
Q	1											20		13			2	16	1	
R														1						
S																				
T																3	2			
V					21		19							8		7				
W																				
X																6				
Y								21	21											
-																				
unknown (?)																	14	17	17	17
not sequenced	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	5	5	5
sum of seq <sup>2</sup>	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	20	17	17	17
oomcaa <sup>3</sup>	19	20	20	21	21	21	19	21	21	21	21	20	14	12	13	7	16	14	17	17
mcaa <sup>4</sup>	E	A	E	D	V	G	V	Y	Y	C	M	Q	A	L	Q	T	P	-	-	-
rel. oomcaa <sup>5</sup>	90%	95%	95%	100%	100%	100%	90%	100%	100%	100%	100%	95%	67%	57%	62%	33%	80%	82%	100%	100%
pos occupied <sup>6</sup>	3	2	2	1	1	1	3	1	1	1	1	2	3	3	3	7	3	3	1	1

Table 4B: Analysis of V kappa subgroup 2

	Framework IV																	
amino acid <sup>1</sup>	E	F	96	97	98	99	100	101	102	103	104	105	106	A	107	108	sum	
A																	71	
B												1					3	
C																	43	
D																	112	
E												13					71	
F			1		17												72	
G					17	2	16					1					233	
H																	26	
I			3										14				94	
K										12					13		66	
L			2								11						219	
M																	37	
N																	56	
P			1														159	
Q			1				14										159	
R										4						12	126	
S																	325	
T				17					16								140	
V											5						146	
W			2														31	
X																	3	
Y			7														123	
-	17	17												13			134	
unknown (?)																	2	
not sequenced <sup>6</sup>	5	5	5	5	5	5	6	6	6	6	6	7	8	9	9	10	211	
sum of seq <sup>7</sup>	17	17	17	17	17	17	16	16	16	16	16	15	14	13	13	12		
oomcaa <sup>1</sup>	17	17	7	17	17	17	14	16	16	12	11	13	14	13	13	12		
mcaa <sup>4</sup>	-	-	Y	T	F	G	Q	G	T	K	L	E	I	-	K	R		
rel. oomcaa <sup>5</sup>	100%	100%	41%	100%	100%	100%	88%	100%	100%	75%	69%	87%	100%	100%	100%	100%		
pos occupied <sup>6</sup>	1	1	7	1	1	1	2	1	1	2	2	3	1	1	1	1		

Table 4C: Analysis of V kappa subgroup 3

Framework I																
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A		5					2	27							1	
B	1															
C												2				
D	2							14								
E	76		27													
F		1													1	
G	1							82							1	152
H										1						
I		75														
K	3															
L		4	1	104			1			150		129			1	
M	5			13												
N															5	
P								124								147
Q						123										
R					1											
S							119		3	1		150		1	141	
T		2			117					147					5	1
V		1	89	1			1				1		22		1	
W																
X																
Y																
-																
unknown (?)																
not sequenced																
sum of seq <sup>2</sup>	88	88	117	118	118	123	123	124	126	149	151	152	152	152	152	152
oomcaa <sup>3</sup>	76	75	89	104	117	123	119	124	82	147	150	150	129	141	147	152
mcaa <sup>4</sup>	E	I	V	L	T	Q	S	P	G	T	L	S	L	S	P	G
rel. oomcaa <sup>5</sup>	86%	85%	76%	88%	99%	100%	97%	100%	65%	99%	99%	99%	85%	93%	97%	100%
pos occupied <sup>6</sup>	6	6	3	3	2	1	4	1	4	3	2	2	3	4	6	1



Table 4C: Analysis of V kappa subgroup 3

	CDRI															
amino acid <sup>1</sup>	17	18	19	20	21	22	23	24	25	26	27	A	B	C	D	E
A			178	2					166	1						
B																
C							181			1						
D	6															
E	146	1									1					
F					7	1										
G	1	1							1	1		1				
H											17					
I		1		5	2											
K		1						5								
L				173							1	1				
M																
N												9				
P																
Q											159					
R		175						176		1	1	10				
S					180				7	175		87				
T		1		174					7	2		1				
V		1	4	1					1			1				
W								1								
X																
Y						1					1					
-												72	182	182	182	182
unknown (?)											1					
not sequenced																
sum of seq <sup>2</sup>	153	181	182	182	182	182	181	182	182	181	181	182	182	182	182	182
oomcaa <sup>3</sup>	146	175	178	174	173	180	181	176	166	175	159	87	182	182	182	182
mcaa <sup>4</sup>	E	R	A	T	L	S	C	R	A	S	Q	S	-	-	-	-
rel. oomcaa <sup>5</sup>	95%	97%	98%	96%	95%	99%	100%	97%	91%	97%	88%	48%	100%	100%	100%	100%
pos occupied <sup>6</sup>	3	7	2	4	3	3	1	3	5	6	6	8	1	1	1	1

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Table 4C: Analysis of V kappa subgroup 3

amino acid <sup>1</sup>	Framework															
	F	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
A				1	1			181								
B																
C																
D			1	1	2	1										
E						1							1			1
F		1				7				1						
G			2	7	3	1		2						1	184	
H			1			2				1		12	1	1		
I		24	4	1	1											
K				1	1								153			
L		8	1			1	176					3				2
M																
N			3	12	25	32										
P					1									170		
Q					1	1				183	167	1				181
R			10	3	18	16		1			1		27	5		
S		72	86	151	118	4								5		
T		1	1	3	8	1										
V		76	68		1		7					3		2		
W			5					185								
X																
Y				1	1	115			183							
-	182															
unknown (?)											1					
not sequenced																
sum of seq <sup>2</sup>	182	182	182	181	181	182	183	184	185	185	185	185	184	184	184	184
oomcaa <sup>1</sup>	182	76	86	151	118	115	176	181	185	183	183	167	153	170	184	181
mcaa <sup>4</sup>	-	V	S	S	S	Y	L	A	W	Y	Q	Q	K	P	G	Q
rel. oomcaa <sup>5</sup>	100%	42%	47%	83%	65%	63%	96%	98%	100%	99%	99%	90%	83%	92%	100%	98%
pos occupied <sup>6</sup>	1	6	11	10	13	12	2	3	1	3	2	4	6	6	1	3

Table 4C: Analysis of V kappa subgroup 3

	CDR II								CDR II							
amino acid <sup>1</sup>	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
A	176							4	147				176	1		
B																
C									1							
D								43					2		4	
E																
F				1		1	4									
G								125					2	10	179	
H							9		1							
I						178								1	168	
K			1								7	1				
L		1		179	174	1										
M						3					1					
N			1					1			53			2		
P	5	184								2			2	2		
Q							1									
R			182					1			4	180				
S							3	6	4	179	74	1		5		
T	3								11	2	44			164		2
V				3	9			3	19				3			15
W							1					1				
X																
Y							165								2	
-																
unknown (?)			1													
not sequenced																
sum of seq <sup>2</sup>	184	185	185	183	183	183	183	183	183	183	183	183	185	185	185	185
oomcaa <sup>3</sup>	176	184	182	179	174	178	165	125	147	179	74	180	176	164	179	168
mcaa <sup>4</sup>	A	P	R	L	L	I	Y	G	A	S	S	R	A	T	G	I
rel. oomcaa <sup>5</sup>	96%	99%	98%	98%	95%	97%	90%	68%	80%	98%	40%	98%	95%	89%	97%	91%
pos occupied <sup>6</sup>	3	2	3	3	2	4	6	7	6	3	6	4	5	7	3	3

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Table 4C: Analysis of V kappa subgroup 3

amino acid <sup>1</sup>	Framework III															
	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
A		68						3		5	3	1		3		
B																
C																
D		112				1						152				
E								1		1		30				
F				183												
G						184	3	178	—	177					2	
H		1														
I				1											1	3
K			1													
L				1												
M								1							182	
N		1												1		
P	177															
Q												1				
R			182		2		1				2					
S	7				180		179		185		3					
T	1		2		3		2							7		2
V		3						1		1						
W										1						
X																
Y																
-													1			
unknown (?)								1								
not sequenced																
sum of seq <sup>1</sup>	185	185	185	185	185	185	185	185	185	185	185	184	184	184	184	184
oomcaa <sup>1</sup>	177	112	182	183	180	184	179	178	185	177	177	152	183	172	182	179
mcaa <sup>1</sup>	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	T
rel. oomcaa <sup>3</sup>	96%	61%	98%	99%	97%	99%	97%	96%	100%	96%	96%	83%	99%	93%	99%	97%
pos occupied <sup>4</sup>	3	5	3	3	3	2	4	5	1	5	4	4	2	5	2	3

Table 4C: Analysis of V kappa subgroup 3

amino acid <sup>1</sup>	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
A							3			174							
B					1												
C									2				1	182			
D			1				3	182									
E					149		175									2	
F		1							178		2	1	4				
G			3					1		2							
H											1				1	7	
I	178							1	1		9						
K							1										
L				178		1			1		7		1			1	
M										1	5						
N	1	5															
P						149											
Q					34										1	181	155
R		1	111							3						1	
S		169	65			34			1				2				
T		8	4							1						8	
V	4			6					1	3	159					7	
W																	
X																	
Y	1										1	183	176		1	2	
-																	
unknown (?)																	
not sequenced																	
sum of seq <sup>2</sup>	184	184	184	184	184	184	182	184	184	184	184	184	184	183	183	183	
oomcaa <sup>3</sup>	178	169	111	178	149	149	175	182	178	174	159	183	176	182	181	155	
mcaa <sup>4</sup>	I	S	R	L	E	P	E	D	F	A	V	Y	Y	C	Q	Q	
rel. oomcaa <sup>5</sup>	97%	92%	60%	97%	81%	81%	96%	99%	97%	95%	86%	99%	96%	99%	99%	85%	
pos occupied <sup>6</sup>	4	5	5	2	3	3	4	3	6	6	7	2	5	2	3	8	

///

Table 4C: Analysis of V kappa subgroup 3

	CDR III															
amino acid <sup>1</sup>	91	92	93	94	95	A	B	C	D	E	F	96	97	98	99	100
A		1	8	3	3											1
B																
C	2			1								2				
D		8	5										1			
E		2										1				
F	5		2									7		166		
G	1	104	15		1	1	2					1			166	41
H	4	1										2				
I			1			1						4				
K			2			1						1				1
L				2	7	5						42				
M		1			1	2										
N		28	71									1				
P				1	139	24						7	2			9
Q	1		1		3	1						3				114
R	34	2	3		2	2						19				
S	2	33	58	102	15	2						1	8			
T		2	13	1	1	2						1	154			
V					3	1						2				
W				69								24				
X																
Y	134	1	1									43				
-			3	3	7	127	167	169	169	169	169	8	1	1	1	1
unknown (?)																
not sequenced						14	14	14	14	14	14	14	17	16	16	16
sum of seq <sup>2</sup>	183	183	183	182	182	169	169	169	169	169	169	169	166	167	167	167
oomcaa <sup>3</sup>	134	104	71	102	139	127	167	169	169	169	169	43	154	166	166	114
mcaa <sup>4</sup>	Y	G	N	S	P	-	-	-	-	-	-	Y	T	F	G	Q
rel. oomcaa <sup>5</sup>	73%	57%	39%	56%	76%	75%	99%	100%	100%	100%	100%	25%	93%	99%	99%	68%
pos occupied <sup>6</sup>	8	11	13	8	11	12	2	1	1	1	1	18	5	2	2	6

Table 4C: Analysis of V kappa subgroup 3

amino acid <sup>1</sup>	Framework IV									sum
	101	102	103	104	105	106	A	107	108	
A										1345
B										2
C										375
D					23					564
E			3		141					759
F						6				765
G	166								1	1804
H					1					64
I						143				803
K			152					157		489
L				54		1			2	1596
M						3				36
N		1						3		255
P		1		1						1147
Q			1		1					1314
R			9			2		4	134	1326
S		2								2629
T		162	1					1		1593
V				111		11				646
W										287
X										
Y			1							1014
-	1	1	1	1	1	1	166	1	1	2151
unknown (?)										4
not sequenced	16	16	15	16	16	16	17	17	45	337
sum of seq <sup>2</sup>	167	167	168	167	167	167	166	166	138	
oomcaa <sup>3</sup>	166	162	152	111	141	143	166	157	134	
mcaa <sup>4</sup>	G	T	K	V	E	I	-	K	R	
rel. oomcaa <sup>5</sup>	99%	97%	90%	66%	84%	86%	100%	95%	97%	
pos occupied <sup>6</sup>	2	5	7	4	5	7	1	5	4	

Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	Framework I																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A												24					1	
B																		
C									1							1		
D	25								26									
E																	25	
F																		
G												1				24		
H																		
I		26																
K						1												
L				1						26					26			
M				24														
N	1																	
P								26				1						
Q			1			25												
R																		26
S							26			25				26		1		
T				26														
V		25	1										26					
W																		
X																		
Y																		
-																		
unknown (?)																		
not sequenced	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
sum of seq <sup>2</sup>	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
oomcaa <sup>3</sup>	25	26	25	24	26	25	26	26	26	25	26	24	26	26	26	24	25	26
mcaa <sup>4</sup>	D	I	V	M	T	Q	S	P	D	S	L	A	V	S	L	G	E	R
rel. oomcaa <sup>5</sup>	96%	100%	96%	92%	100%	96%	100%	100%	100%	96%	100%	92%	100%	100%	100%	92%	96%	100%
pos occupied <sup>6</sup>	2	1	2	3	1	2	1	1	1	2	1	3	1	1	1	3	2	1



Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	CDRI																	
	19	20	21	22	23	24	25	26	27	A	B	C	D	E	F	28	29	30
A	26						1				1							
B																		
C					33													
D											1		1			1		
E																		
F																		
G																		
H																		
I			26								1							
K						33										2		30
L											2	31						
M																		
N				26												30	31	1
P							1								1			
Q									32									1
R									1								1	1
S							31	33		33				32	32		1	
T		26													1			
V											28	2						
W																		
X																		
Y													32					
-																		
unknown (?)																		
not sequenced	7	7	7	7														
sum of seq <sup>2</sup>	26	26	26	26	33	33	33	33	33	33	33	33	33	33	33	33	33	33
oomcaa <sup>3</sup>	26	26	26	26	33	33	31	33	32	33	28	31	32	32	32	30	31	30
mcaa <sup>4</sup>	A	T	I	N	C	K	S	S	Q	S	V	L	Y	S	S	N	N	K
rel. oomcaa <sup>5</sup>	100%	100%	100%	100%	100%	100%	94%	100%	97%	100%	85%	94%	97%	97%	97%	91%	94%	91%
pos occupied <sup>6</sup>	1	1	1	1	1	1	3	1	2	1	5	2	2	2	2	3	3	4

Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	Framework II																	
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
A				32						2								
B																		
C																		
D																		
E											1							
F																		
G										32								
H						2												
I																		32
K									33						32			
L			33													29	33	
M																		1
N	33																	
P										31			31	33				
Q							32	33				32						
R							1					1				1		
S														2				
T				1														
V																4		
W					33													
X																		
Y		33				31												
-																		
unknown (?)																		
not sequenced																		
sum of seq <sup>2</sup>	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
oomcaa <sup>3</sup>	33	33	33	32	33	31	32	33	33	31	32	32	31	33	32	29	33	32
mcaa <sup>4</sup>	N	Y	L	A	W	Y	Q	Q	K	P	G	Q	P	P	K	L	L	I
rel. oomcaa <sup>5</sup>	100%	100%	100%	97%	100%	94%	97%	100%	100%	94%	97%	97%	94%	100%	97%	88%	100%	97%
pos occupied <sup>6</sup>	1	1	1	2	1	2	2	1	1	2	2	2	2	1	2	2	1	2

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Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	CDR II																	
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
A			30															
B																		
C																		
D												33						
E							32											
F														33				
G									33						1	33		33
H																		
I					1													
K																		
L																		
M																		
N					2													
P				1							33		1					
Q																		
R						33								32				
S			1	31	1			33							32		33	
T			2	1	29													
V							1			33								
W		33																
X																		
Y	33																	
-																		
unknown (?)																		
not sequenced																		
sum of seq <sup>1</sup>	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
oomcaa <sup>1</sup>	33	33	30	31	29	33	32	33	33	33	33	33	32	33	32	33	33	33
mcaa <sup>4</sup>	Y	W	A	S	T	R	E	S	G	V	P	D	R	F	S	G	S	G
rel. oomcaa <sup>5</sup>	100%	100%	91%	94%	88%	100%	97%	100%	100%	100%	100%	100%	97%	100%	97%	100%	100%	100%
pos occupied <sup>6</sup>	1	1	3	3	4	1	2	1	1	1	1	1	2	1	2	1	1	1

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Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	Framework III																	
	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
A														33				32
B																		
C																		
D				32												33		
E															33			
F					32													
G		33		1														1
H																		
I									33									
K																		
L							33					32						
M												1						
N										2	1							
P																		
Q													32					
R													1					
S	33									30	32							
T		33				33		33		1								
V					1												33	
W																		
X																		
Y																		
-																		
unknown (?)																		
not sequenced																		
sum of seq <sup>2</sup>	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
oomcaa <sup>3</sup>	33	33	33	32	32	33	33	33	33	30	32	32	32	33	33	33	33	32
mcaa <sup>4</sup>	S	G	T	D	F	T	L	T	I	S	S	L	Q	A	E	D	V	A
rel. oomcaa <sup>5</sup>	100%	100%	100%	97%	97%	100%	100%	100%	100%	91%	97%	97%	97%	100%	100%	100%	100%	97%
pos occupied <sup>6</sup>	1	1	1	2	2	1	1	1	1	3	2	2	2	1	1	1	1	2

Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	CDR III																
	85	86	87	88	89	90	91	92	93	94	95	A	B	C	D	E	F
A										1							
B																	
C				33													
D								1	1								
E																	
F			1					1									
G									2	1							
H			1		3												
I										2							
K																	
L						1		2		1	3						1
M																	
N									4	4							
P										1	29	1					4
Q					30	32					1						1
R									1			1					2
S							2		23	2							1
T									2	22							
V	33																
W																	2
X																	
Y		33	31				31	29									1
-												13	15	15	15	15	3
unknown (?)																	
not sequenced												18	18	18	18	18	18
sum of seq <sup>2</sup>	33	33	33	33	33	33	33	33	33	33	33	15	15	15	15	15	15
oomcaa <sup>3</sup>	33	33	31	33	30	32	31	29	23	22	29	13	15	15	15	15	4
mcaa <sup>4</sup>	V	Y	Y	C	Q	Q	Y	Y	S	T	P	-	-	-	-	-	P
rel. oomcaa <sup>5</sup>	100%	100%	94%	100%	91%	97%	94%	88%	70%	67%	88%	87%	100%	100%	100%	100%	27%
pos occupied <sup>6</sup>	1	1	3	1	2	2	2	4	6	7	3	3	1	1	1	1	8

Table 4D: Analysis of V kappa subgroup 4

amino acid <sup>1</sup>	Framework IV												sum
	97	98	99	100	101	102	103	104	105	106	A	107	108
A													183
B													
C													68
D													154
E									14				105
F		15											82
G			15	4	15								228
H													6
I										14			135
K							14					13	158
L								4					258
M	1												27
N												1	136
P						1							195
Q				11				1					264
R							1		1			1	116
S	2									1			499
T	12					14							236
V								9					196
W								1					69
X													
Y													254
-										15			106
unknown (?)													
not sequenced	18	18	18	18	18	18	18	18	18	18	18	18	22
sum of seq <sup>2</sup>	15	15	15	15	15	15	15	15	15	15	15	15	11
oomcaa <sup>3</sup>	12	15	15	11	15	14	14	9	14	14	15	13	11
mcaa <sup>4</sup>	T	F	G	Q	G	T	K	V	E	I	-	K	R
rel. oomcaa <sup>5</sup>	80%	100%	100%	73%	100%	93%	93%	60%	93%	93%	100%	87%	100%
pos occupied <sup>6</sup>	3	1	1	2	1	2	2	4	2	2	1	3	1

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Table 5A: Analysis of V lambda subgroup 1

amino acid <sup>1</sup>	Framework I																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A											19		18	20					
B																			
C																			
D																			
E																		1	
F																			
G													22			42			
H	2																		
I			1								1								
K																		14	
L			1	41							1								
M																			
N																			
P							41	41						1	41				
Q	22		1			41												42	
R																		25	
S		39							41			41			1			1	
T					41									19				1	
V		1	38								20		1	1					42
W																			
X																			
Y																			
Z	16																		
-										41									
unknown (?)																			
not sequenced	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1				
sum of seq <sup>2</sup>	40	40	41	41	41	41	41	41	41	41	41	41	41	41	42	42	42	42	42
oomcaa <sup>1</sup>	22	39	38	41	41	41	41	41	41	41	20	41	22	20	41	42	42	25	42
mcaa <sup>1</sup>	Q	S	V	L	T	Q	P	P	S	-	V	S	G	A	P	G	Q	R	V
rel. oomcaa <sup>3</sup>	55%	98%	93%	100%	100%	100%	100%	100%	100%	100%	49%	100%	54%	49%	98%	100%	100%	60%	100%
pos occupied <sup>4</sup>	3	2	4	1	1	1	1	1	1	1	4	1	3	4	2	1	1	5	1

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Table 5A: Analysis of V lambda subgroup 1

PCT/EP96/03647

amino acid <sup>1</sup>	CDRI																		
	20	21	22	23	24	25	26	27	D	E	28	29	30	31	A	32	33	34	35
A	2							1				2	2			1			
B																			
C				42															
D										3			3	1		3		1	
E													1						
F					1				1						1	1			
G					42	3	1				2	39	4	2					
H														2		2		2	
I	1	41								1	37							1	
K										1			1						
L		1									1								
M											1								
N								2	1	37			13	31	2		1	9	
P																1			
Q																1			
R						1	1						5						
S	1	42		38		34	34	38					13	1	1	3		19	
T	38			3		4	3	2				1		1		7		2	
V											1					2	40		
W																			42
X																			
Y														4	1	20		7	
Z																			
-															36				
unknown (?)																			
not sequenced															1	1	1	1	
sum of seq <sup>2</sup>	42	42	42	42	42	42	42	42	42	42	42	42	42	42	41	41	41	41	42
oomcaa <sup>3</sup>	38	41	42	42	38	42	34	34	38	37	37	39	13	31	36	20	40	19	42
mcaa <sup>4</sup>	T	I	S	C	S	G	S	S	S	N	I	G	N	N	-	Y	V	S	W
rel. oomcaa <sup>5</sup>	90%	98%	100%	100%	90%	100%	81%	81%	90%	88%	88%	93%	31%	74%	88%	49%	98%	46%	100%
pos occupied <sup>6</sup>	4	2	1	1	3	1	4	6	4	4	5	3	8	7	5	10	2	7	1



Table 5A: Analysis of V lambda subgroup 1

	Framework II																			
amino acid <sup>1</sup>	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
A							4	40										1		
B																				
C																				
D						1									13	10	8			
E										2					5			1		
F	1			4										1						
G						39									1					
H	1	1	6	1										1				1		
I													40		1					
K							1			35					1	1		18		
L			1	31							41	40						1	1	
M							1							1				1		
N										1					3	28	30	2		
P					42	1			42											
Q		39	34															15		
R		2		1		1				4					7			2	40	
S								1							9	2	3	1		
T							36	1							1					
V			1	5							1	2	1							
W																			1	
X																				
Y	40													40	1	1				
Z																				
-																				
unknown (?)																				
not sequenced																				
sum of seq <sup>2</sup>	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	
oomcaa <sup>3</sup>	40	39	34	31	42	39	36	40	42	35	41	40	40	40	13	28	30	18	40	
mcaa <sup>4</sup>	Y	Q	Q	L	P	G	T	A	P	K	L	L	I	Y	D	N	N	K	R	
rel. oomcaa <sup>5</sup>	95%	93%	81%	74%	100%	93%	86%	95%	100%	83%	98%	95%	95%	95%	31%	67%	71%	43%	95%	
pos occupied <sup>6</sup>	3	3	4	5	1	4	4	3	1	4	2	2	3	3	10	5	4	9	3	

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Table 5A: Analysis of V lambda subgroup 1

		CDR II																	
amino acid <sup>1</sup>	55	56	A	B	C	D	E	57	58	59	60	61	62	63	64	65	66	A	B
A	1														5				
B																			
C																			
D											38								
E																			
F													38						
G								41			2				36				
H											1								
I									17				3						
K																	38		
L		1								1									
M																			
N																			
P	38									38									
Q																			
R												42					4		
S	2	40								2				42		42			
T															1				
V									24				1						
W																			
X																			
Y																			
Z																			
-			41	41	41	41	42											42	42
unknown (?)																			
not sequenced	1	1						1	1	1	1								
sum of seq <sup>2</sup>	41	41	41	41	41	41	42	41	41	41	41	42	42	42	42	42	42	42	42
oomcaa <sup>3</sup>	38	40	41	41	41	41	42	41	24	38	38	42	38	42	36	42	38	42	42
mcaa <sup>4</sup>	P	S	-	-	-	-	-	G	V	P	D	R	F	S	G	S	K	-	-
rel. oomcaa <sup>5</sup>	93%	98%	100%	100%	100%	100%	100%	100%	59%	93%	93%	100%	90%	100%	86%	100%	90%	100%	100%
pos occupied <sup>6</sup>	3	2	1	1	1	1	1	1	2	3	3	1	3	1	3	1	2	1	1

Table 5A: Analysis of V lambda subgroup 1

amino acid <sup>1</sup>	Framework III																		
	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
A		1	3		41			24						2				38	1
B																			
C																			
D		1													1	41			37
E													1		24		42		1
F																			
G		40						17		1	42				15				
H													1						2
I									41										1
K																			
L							42					41							
M																			
N																1			
P														2					
Q													31						
R													8						
S	42		1	42		24				20				20					1
T			38			18				21				17					3
V					1			1	1			1		1					
W													1		2				
X																			
Y																			
Z																			
-																			
unknown (?)																			
not sequenced																			
sum of seq <sup>2</sup>	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
oomcaa <sup>3</sup>	42	40	38	42	41	24	42	24	41	21	42	41	31	20	24	41	42	38	37
mcaa <sup>4</sup>	S	G	T	S	A	S	L	A	I	T	G	L	Q	S	E	D	E	A	D
rel. oomcaa <sup>5</sup>	100%	95%	90%	100%	98%	57%	100%	57%	98%	50%	100%	98%	74%	48%	57%	98%	100%	90%	88%
pos occupied <sup>6</sup>	1	3	3	1	2	2	1	3	2	3	1	2	5	5	4	2	1	3	5

Table 5A: Analysis of V lambda subgroup 1

	CDR III																		
amino acid <sup>1</sup>	86	87	88	89	90	91	92	93	94	95	A	B	C	D	E	F	96	97	98
A				22	15			1				16					4	1	
B																			
C			42																
D							39	17			7								
E												1					1		
F		2								1									36
G				14				1				17	1				5	1	
H		1											1						
I											1							1	
K											1								
L				1						37			1					1	
M																		1	
N							2	2			9	1							
P										1							6		
Q				3															
R									5	1	2						2		
S					4			17	35		18		1				1		
T				22				1	1		1								
V				1				1		1		2					9	34	
W						38											7		
X																			
Y	42	39				3		1									3		
Z																			
-											2	4	35	39	38	38	1		
unknown (?)																			
not sequenced				1	1	1	1	1	1	1	1	1	3	3	3	3	3	3	4
sum of seq <sup>2</sup>	42	42	42	41	41	41	41	41	41	41	41	41	39	39	38	38	39	39	36
oomcaa <sup>3</sup>	42	39	42	22	22	38	39	17	35	37	18	17	35	39	38	38	9	34	36
mcaa <sup>4</sup>	Y	Y	C	A	T	W	D	D	S	L	S	G	-	-	-	-	V	V	F
rel. oomcaa <sup>5</sup>	100%	93%	100%	54%	54%	93%	95%	41%	85%	90%	44%	41%	90%	100%	100%	100%	23%	87%	100%
pos occupied <sup>6</sup>	1	3	1	5	3	2	2	8	3	5	8	6	5	1	1	1	10	6	1

Table 5A: Analysis of V lambda subgroup 1

	Framework IV												
amino acid <sup>1</sup>	99	100	101	102	103	104	105	106	A	107	108	sum	
A												285	
B													
C												84	
D												224	
E		1										81	
F												87	
G	36	31	36							26		559	
H												25	
I												188	
K					30							141	
L						25			34			344	
M												5	
N					1							176	
P											1	296	
Q					3				1	18		251	
R					1					2		156	
S		1								2		720	
T		3		36	1		36					359	
V						11		36	1			282	
W										1		92	
X													
Y												202	
Z												16	
-												524	
unknown (?)													
not sequenced <sup>2</sup>	4	6	6	6	6	6	6	6	6	10	22	141	
sum of seq <sup>3</sup>	36	36	36	36	36	36	36	36	36	31	19		
oomcaa <sup>3</sup>	36	31	36	36	30	25	36	36	34	26	18		
mcaa <sup>4</sup>	G	G	G	T	K	L	T	V	L	G	Q		
rel. oomcaa <sup>5</sup>	100%	86%	100%	100%	83%	69%	100%	100%	94%	84%	95%		
pos occupied <sup>6</sup>	1	4	1	1	5	2	1	1	3	4	2		

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Table 5B: Analysis of V lambda subgroup 2

amino acid <sup>1</sup>	Framework I																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A			35					30			6		1	1					
B																			
C																			
D																1			
E																			
F																			
G												42				42			
H	2																1		
I			1																28
K																			
L				40											3				1
M																			
N																			
P							42	6							40				
Q	22		4			41											42		
R								6	1										
S		41						40			42		42					43	
T				42				1											
V		1	2							36									14
W																			
X																			
Y																			
Z	16																		
-									42										
unknown (?)						1													
not sequenced	3	1	1	3	1	1	1	1	1	1	1	1							
sum of seq <sup>2</sup>	40	42	42	40	42	42	42	42	42	42	42	42	43	43	43	43	43	43	43
oomcaa <sup>3</sup>	22	41	35	40	42	41	42	30	40	42	36	42	42	42	40	42	42	43	28
mcaa <sup>4</sup>	Q	S	A	L	T	Q	P	A	S	-	V	S	G	S	P	G	Q	S	I
rel. oomcaa <sup>5</sup>	55%	98%	83%	100%	100%	98%	100%	71%	95%	100%	86%	100%	98%	98%	93%	98%	98%	100%	65%
pos occupied <sup>6</sup>	3	2	4	1	1	1	1	3	3	1	2	1	2	2	2	2	2	1	3

Table 5B: Analysis of V lambda subgroup 2

	CDRI																				
amino acid <sup>1</sup>	20	21	22	23	24	25	26	27	D	E	28	29	30	31	A	32	33	34	35		
A					3		1						1			1					
B																					
C				42					1					1							
D										39		1	4			5					
E																1					
F		1											1				4				
G						43		1				39	26								
H								1								1	1				
I		41			1						6										
K																4					
L		1															4				
M																					
N								1	3	4		1	4	3	28						
P								1													
Q																					
R									1				2								
S			42		3		3	35	38				5	1	2	4	1	42			
T	43				36		39	3				1		1							
V											37						41				
W																			43		
X																					
Y								1				1		37		29					
Z																					
-																1					
unknown (?)																1					
not sequenced			1	1														1	1		
sum of seq <sup>2</sup>	43	43	42	42	43	43	43	43	43	43	43	43	43	43	43	43	42	42	43		
oomcaa <sup>3</sup>	43	41	42	42	36	43	39	35	38	39	37	39	26	37	28	29	41	42	43		
mcaa <sup>4</sup>	T	I	S	C	T	G	T	S	S	D	V	G	G	Y	N	Y	V	S	W		
rel. oomcaa <sup>5</sup>	100%	95%	100%	100%	84%	100%	91%	81%	88%	91%	86%	91%	60%	86%	65%	67%	98%	100%	100%		
pos occupied <sup>6</sup>	1	3	1	1	4	1	3	7	4	2	2	5	7	5	7	6	2	1	1		

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Table 5B: Analysis of V lambda subgroup 2

amino acid <sup>1</sup>	Framework II																							
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54					
A					1	4		40																
B																								
C																								
D				1		2									20	1	2	1						
E															20				2					
F	2														7		1							
G						36										2	2		1					
H			2	34															1					
I							1				1	9	43					1						
K						40				41								1	21					
L			1	1							38	6												
M												26						1						
N				2											1		8	12						
P					41				43															
Q		41	39							2														
R		1					1																	
S					1										2			21	3					
T							1											7						
V						1		3			4	2				39								
W																								
X																								
Y	41			5											34				2					
Z																								
-																								
unknown (?)		1	1																					
not sequenced																								
sum of seq <sup>2</sup>	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
oomcaa <sup>1</sup>	41	41	39	34	41	36	40	40	43	41	38	26	43	34	20	39	21	21	43					
mcaa <sup>1</sup>	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	Y	D	V	S	K	R					
rel. oomcaa <sup>3</sup>	95%	95%	91%	79%	95%	84%	93%	93%	100%	95%	88%	60%	100%	79%	47%	91%	49%	49%	100%					
pos occupied <sup>4</sup>	2	2	3	5	3	4	4	2	1	2	3	4	1	3	4	4	8	8	1					



Table 5B: Analysis of V lambda subgroup 2

amino acid <sup>1</sup>	CDR II																			A	B
	55	56	A	B	C	D	E	57	58	59	60	61	62	63	64	65	66				
A															2						
B																					
C																1					
D											17										
E																					
F													42								
G								43	1						41						
H											2										
I									3												
K																42					
L											1		1								
M																					
N											19										
P	43									15											
Q																					
R												43					1				
S		43								28	2			43		42					
T																					
V									39												
W																					
X																					
Y											2										
Z																					
-			43	43	43	43	43												43	43	
unknown (?)																					
not sequenced																					
sum of seq <sup>2</sup>	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
oomcaa <sup>3</sup>	43	43	43	43	43	43	43	43	39	28	19	43	42	43	41	42	42	43	43		
mcaa <sup>4</sup>	P	S	-	-	-	-	-	G	V	S	N	R	F	S	G	S	K	-	-		
rel. oomcaa <sup>5</sup>	100%	100%	100%	100%	100%	100%	100%	100%	91%	65%	44%	100%	98%	100%	95%	98%	98%	100%	100%		
pos occupied <sup>6</sup>	1	1	1	1	1	1	1	1	3	2	6	1	2	1	2	2	2	1	1		

Table 5B: Analysis of V lambda subgroup 2

Framework III																				
amino acid <sup>1</sup>	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	
A		3		1	43									36				43		
B																				
C																				
D		1	2												3	42			39	
E											1				38		43			
F																				
G		39									42				1					
H																			2	
I									35											
K			1																	
L						43						43								
M																				
N			38													1	1		1	
P																				
Q													41							
R													2							
S	42			1		43				42										
T			1	41				43		1				2						
V									8					3						
W																				
X																				
Y																				
Z																				
-																				
unknown (?)			1																	
not sequenced	1																		1	
sum of seq <sup>2</sup>	42	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	
oomcaa <sup>3</sup>	42	39	38	41	43	43	43	43	35	42	42	43	41	36	38	42	43	43	39	
mcaa <sup>4</sup>	S	G	N	T	A	S	L	T	I	S	G	L	Q	A	E	D	E	A	D	
rel. oomcaa <sup>5</sup>	100%	91%	88%	95%	100%	100%	100%	100%	81%	98%	98%	100%	95%	84%	88%	98%	100%	100%	91%	
pos occupied <sup>6</sup>	1	3	4	3	1	1	1	1	2	2	2	1	2	4	4	2	1	1	3	

Table 5B: Analysis of V lambda subgroup 2

	CDR III																		
amino acid <sup>1</sup>	86	87	88	89	90	91	92	93	94	95	A	B	C	D	E	F	96	97	98
A				2	1		21		1								1	1	
B																			
C			43	11															
D								3	1	2							1		
E							1	1											
F		3				3				1		1					5		42
G							1	21	3	4							1		
H						1													
I							1	1		1	2						1	7	
K										3									
L												1	1				6	5	
M																	1	1	
N									5	7	5						1		
P								1					4						
Q										1	2								
R							2		3				1				5		
S		1		30	41			12	23	14	9						1		
T							16	4	4	3	21								
V							1										11	28	
W																	5		
X																			
Y	43	39				39			1	6							4		
Z																			
-										1	3	36	42	43	43	43			
unknown (?)									2										
not sequenced					1						1							1	1
sum of seq <sup>2</sup>	43	43	43	43	42	43	43	43	43	43	42	43	43	43	43	43	43	42	42
oomcaa <sup>3</sup>	43	39	43	30	41	39	21	21	23	14	21	36	42	43	43	43	11	28	42
mcaa <sup>4</sup>	Y	Y	C	S	S	Y	A	G	S	S	T	-	-	-	-	-	V	V	F
rel. oomcaa <sup>5</sup>	100%	91%	100%	70%	98%	91%	49%	49%	53%	33%	50%	84%	98%	100%	100%	100%	26%	67%	100%
pos occupied <sup>6</sup>	1	3	1	3	2	3	7	7	8	11	6	5	2	1	1	1	13	5	1

Table 5B: Analysis of V lambda subgroup 2

amino acid <sup>1</sup>	Framework IV										sum
	99	100	101	102	103	104	105	106	A	107	108
A		1									280
B											
C											99
D											188
E											107
F											113
G	42	33	42							19	567
H											48
I							1				184
K					36						189
L						28			40		264
M											29
N					1						146
P											238
Q					1					14	250
R		1			2					4	121
S							1			2	831
T		7		41			40				398
V						14		42	1		327
W											48
X											
Y					1						285
Z											16
-											555
unknown (?)											8
not sequenced	1	1	1	2	2	1	1	1	2	15	80
sum of seq <sup>2</sup>	42	42	42	41	41	42	42	42	41	25	14
oomcaa <sup>3</sup>	42	33	42	41	36	28	40	42	40	19	14
mcaa <sup>4</sup>	G	G	G	T	K	L	T	V	L	G	Q
rel. oomcaa <sup>5</sup>	100%	79%	100%	100%	88%	67%	95%	100%	98%	76%	100%
pos occupied <sup>6</sup>	1	4	1	1	5	2	3	1	2	3	1

Table 5C: Analysis of V lambda subgroup 3

Framework I																			
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A					1		1	2	7					20	1				27
B																			
C																			
D			5				10												
E			20										1			1			
F	1	1										1			1				
G			1													37			
H																			
I																			
K																	2		
L				37							4		1		9				
M																			
N																			
P							26	35	1						27				1
Q	4		4			38											36		
R																			
S	13	14			1		1		28			37		18					
T					36			1										38	
V			8	1					2		34		36						10
W																			
X																			
Y		23																	
Z																			
-	20									38									
unknown (?)																			
not sequenced																			
sum of seq <sup>2</sup>	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
oomcaa <sup>3</sup>	20	23	20	37	36	38	26	35	28	38	34	37	36	20	27	37	36	38	27
mcaa <sup>4</sup>	-	Y	E	L	T	Q	P	P	S	-	V	S	V	A	P	G	Q	T	A
rel. oomcaa <sup>5</sup>	53%	61%	53%	97%	95%	100%	68%	92%	74%	100%	89%	97%	95%	53%	71%	97%	95%	100%	71%
pos occupied <sup>6</sup>	4	3	5	2	3	1	4	3	4	1	2	2	3	2	4	2	2	1	3

Table 5C: Analysis of V lambda subgroup 3

amino acid <sup>1</sup>	CDRI															
	20	21	22	23	24	25	26	27	D	E	28	29	30	31	A	32
A			1					5					1	1		21
B																3
C				38												5
D							30	1					10			3
E							2	2				1	3	6		1
F														1		2
G					9	38		1				23	4			
H							1									2
I		38									9			1		9
K								7					2	13		
L											28					
M	1													1		
N			2				4	9			1		2			1
P			1									3				2
Q					10									4		
R	25							2				10	1			1
S	9		1		19			10					11	2		8
T	3		33					1				1	4			14
V																1
W																15
X																38
Y							1							8		20
Z																1
-									38	38					37	
unknown (?)																
not sequenced															1	1
sum of seq <sup>2</sup>	38	38	38	38	38	38	38	38	38	38	38	38	38	37	37	37
oomcaa <sup>3</sup>	25	38	33	38	19	38	30	10	38	38	28	23	11	13	37	20
mcaa <sup>4</sup>	R	I	T	C	S	G	D	S	-	-	L	G	S	K	-	Y
rel. oomcaa <sup>5</sup>	66%	100%	87%	100%	50%	100%	79%	26%	100%	100%	74%	61%	29%	35%	100%	54%
pos occupied <sup>6</sup>	4	1	5	1	3	1	5	9	1	1	3	5	9	9	1	7

Table 5C: Analysis of V lambda subgroup 3

	Framework II																							
amino acid <sup>1</sup>	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54					
A								23								1			1					
B																								
C																								
D															9	22	2	8						
E			1												5	3		3						
F	3													2			1							
G						36									9	2								
H							1							1	3			1						
I										1		28					1							
K				32											2	6	1	13						
L			2							6	33	1												
M											1		1											
N																1	19	9						
P					36		1		38															
Q		37	35	1			36								9			1						
R		1		4		2									1	1		1	38					
S				1	2			14									10	1						
T																2	4							
V							1		31	4	37	9												
W																								
X																								
Y	35													35										
Z																								
-																								
unknown (?)																								
not sequenced																								
sum of seq <sup>2</sup>	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38					
oomcaa <sup>1</sup>	35	37	35	32	36	36	36	23	38	31	33	37	28	35	9	22	19	13	38					
mcaa <sup>1</sup>	Y	Q	Q	K	P	G	Q	A	P	V	L	V	I	Y	D	D	N	K	R					
rel. oomcaa <sup>5</sup>	92%	97%	92%	84%	95%	95%	95%	61%	100%	82%	87%	97%	74%	92%	24%	58%	50%	34%	100%					
pos occupied <sup>6</sup>	2	2	3	4	2	2	3	3	1	3	3	2	3	3	7	8	7	9	1					

Table 5C: Analysis of V lambda subgroup 3

		CDR II																			
amino acid <sup>1</sup>	55	56	A	B	C	D	E	57	58	59	60	61	62	63	64	65	66	A	B		
A		1																			
B																					
C																					
D											9										
E											27										
F													38								
G								38							38						
H																					
I									37												
K																					
L																					
M																					
N																	21				
P	37	1								36											
Q																					
R												38									
S	1	36								1				38		38	12				
T																	5				
V																					
W																					
X																					
Y																					
Z																					
-			38	38	38	38	38											38	38		
unknown (?)											1										
not sequenced									1	1	1										
sum of seq <sup>2</sup>	38	38	38	38	38	38	38	38	37	37	37	38	38	38	38	38	38	38	38		
oomcaa <sup>3</sup>	37	36	38	38	38	38	38	38	37	36	27	38	38	38	38	38	21	38	38		
mcaa <sup>4</sup>	P	S	-	-	-	-	-	G	I	P	E	R	F	S	G	S	N	-	-		
rel. oomcaa <sup>5</sup>	97%	95%	100%	100%	100%	100%	100%	100%	100%	97%	73%	100%	100%	100%	100%	100%	55%	100%	100%		
pos occupied <sup>6</sup>	2	3	1	1	1	1	1	1	1	2	2	1	1	1	1	1	3	1	1		



Table 5C: Analysis of V lambda subgroup 3

	Framework III																			
amino acid <sup>1</sup>	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	
A				1	36	1		1				11	1	34				38		
B																				
C																				
D																38			37	
E													10		14		38		1	
F																				
G		37									28				10					
H			1																	
I						1		1	37	1						1				
K			1																	
L							38									2				
M															10					
N			28							1										
P																				
Q		1											25							
R										1	10		1							
S	37		2			11				23				1						
T	1		6	37		25		36		12		13		2						
V					2				1			14	1	1	1					
W																				
X																				
Y																				
Z																				
-																				
unknown (?)																				
not sequenced																				
sum of seq <sup>2</sup>	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	
oomcaa <sup>3</sup>	37	37	28	37	36	25	38	36	37	23	28	14	25	34	14	38	38	38	37	
mcaa <sup>4</sup>	S	G	N	T	A	T	L	T	I	S	G	V	Q	A	E	D	E	A	D	
rel. oomcaa <sup>5</sup>	97%	97%	74%	97%	95%	66%	100%	95%	97%	61%	74%	37%	66%	89%	37%	100%	100%	100%	97%	
pos occupied <sup>6</sup>	2	2	5	2	2	4	1	3	2	5	2	3	5	4	6	1	1	1	2	

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Table 5C: Analysis of V lambda subgroup 3

	CDR III																		
amino acid <sup>1</sup>	86	87	88	89	90	91	92	93	94	95	A	B	C	D	E	F	96	97	98
A					13	3	2			1	2						4		
B																			
C			38																
D							32	1	1		6								
E				1								2					2		
F		2						2											35
G									3	14	3			1			3	1	
H												12	1						
I																		4	
K											1								
L				1				1		1		1	1				4	2	
M									1								1	1	
N				10			2	1	2		10	1							
P									1				3				1		
Q				25						1	1								
R					10			1	2			2							
S				1	14	1		28	26	13		1				1			
T						1		3		7	2								
V					11												18	28	
W						23											1		
X																			
Y	38	36					1		1		1	3	1				3		
Z																			
-											10	15	31	36	37	36		1	
unknown (?)																			
not sequenced							1	1	1	1	2	1	1	1	1	1	1	1	3
sum of seq <sup>2</sup>	38	38	38	38	38	38	37	37	37	37	36	37	37	37	37	37	37	37	35
oomcaa <sup>1</sup>	38	36	38	25	14	23	32	28	26	14	10	15	31	36	37	36	18	28	35
mcaa <sup>1</sup>	Y	Y	C	Q	S	W	D	S	S	G	N	-	-	-	-	-	V	V	F
rel. oomcaa <sup>5</sup>	100%	95%	100%	66%	37%	61%	86%	76%	70%	38%	28%	41%	84%	97%	100%	97%	49%	76%	100%
pos occupied <sup>6</sup>	1	2	1	5	3	5	4	7	8	6	9	8	5	2	1	2	9	6	1

Table 5C: Analysis of V lambda subgroup 3

amino acid <sup>1</sup>	Framework IV											sum
	99	100	101	102	103	104	105	106	A	107	108	
A												265
B												
C										1		82
D												225
E					2							145
F												90
G	35	31	35							24		461
H												32
I												160
K					30							110
L						28			33			233
M												17
N												126
P									1			249
Q											7	275
R					2							154
S										2		501
T		4		35			35					347
V						7		35				308
W												62
X												
Y												211
Z												
-												603
unknown (?)												1
not sequenced	3	3	3	3	4	3	3	3	4	11	28	89
sum of seq <sup>2</sup>	35	35	35	35	34	35	35	35	34	27	7	
oomcaa <sup>3</sup>	35	31	35	35	30	28	35	35	33	24	7	
mcaa <sup>4</sup>	G	G	G	T	K	L	T	V	L	G	Q	
rel. oomcaa <sup>5</sup>	100%	89%	100%	100%	88%	80%	100%	100%	97%	89%	100%	
pos occupied <sup>6</sup>	1	2	1	1	3	2	1	1	2	3	1	

Table 6A: Analysis of V heavy chain subgroup 1A

amino acid <sup>1</sup>	Framework I																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A					1	14			60							24	1			
B																				
C																				
D																				
E	1				2	1		2		64										
F																				
G								58	1						64					
H			2																	
I		2																		
K		2										57	64						60	
L			2	59							3									
M		1																		
N												6								
P														63						
Q	53		56		2	45														
R												1							3	
S						60		3						1		40	63			
T																			1	
V	2	55		1	55					61								64		64
W																				
X																				
Y																				
Z	3																			
-																				
unknown (?)																				
not sequenced	11	10	10	10	10	10	10	10	6	6	6	6	6	6	6	6	6	6	6	6
sum of seq <sup>2</sup>	59	60	60	60	60	60	60	60	64	64	64	64	64	64	64	64	64	64	64	64
oomcaa <sup>3</sup>	53	55	56	59	55	45	60	58	60	64	61	57	64	63	64	40	63	64	60	64
mcaa <sup>4</sup>	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	S	V	K	V
rel. oomcaa <sup>5</sup>	90%	92%	93%	98%	92%	75%	100%	97%	94%	100%	95%	89%	100%	98%	100%	63%	98%	100%	94%	100%
pos occupied <sup>6</sup>	4	4	3	2	4	3	1	2	3	1	2	3	1	2	1	2	2	1	3	1

Table 6A: Analysis of V heavy chain subgroup 1A

																					CDRI			
amino acid <sup>1</sup>	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38				
A				62				1							41									
B																								
C		63																						
D							1																	
E																								
F									69					3		3								
G				1		69	41		1						23									
H										1				1			1							
I								1								61	1		1					
K			63							1	1													
L																1	2							
M																	4							
N										2	5							4						
P																1								
Q																								
R		1	1							1	1												70	
S	63				68		1			40	60			2			60							
T	1			2				68		25	3					3		4						
V															1							69		
W																		70						
X																								
Y							27								64									
Z																								
-												70	70											
unknown (?)																								
not sequenced	6	6	6	5	2	1																		
sum of seq <sup>2</sup>	64	64	64	65	68	69	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
oomcaa <sup>3</sup>	63	63	63	62	68	69	41	68	69	40	60	70	70	64	41	61	60	70	69	70				
mcaa <sup>4</sup>	S	C	K	A	S	G	G	T	F	S	S	-	-	Y	A	I	S	W	V	R				
rel. oomcaa <sup>5</sup>	98%	98%	98%	95%	100%	100%	59%	97%	99%	57%	86%	100%	100%	91%	59%	87%	86%	100%	99%	100%				
pos occupied <sup>6</sup>	2	2	2	3	1	1	4	3	2	6	5	1	1	4	6	4	5	1	2	1				

Table 6A: Analysis of V heavy chain subgroup 1A

amino acid <sup>1</sup>	Framework II																			
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55
A		70									1				5					
B																				
C																				
D								1												
E								69												
F														2				3	39	
G			1	68		69			1		69	39			1					68
H			1																	
I													65	38				34		
K																				
L				1			68			1		1						2	4	
M									67					2				4		
N														4				3	22	
P			68				1								44					
Q	69				69													1	1	1
R	1			1		1						4						1		
S					1				1	1				22					1	1
T													1	2	4			1	3	
V										1			2	2	16			1		
W							1	67				26								
X																				
Y									1									20		
Z																				
-																70	70			
unknown (?)																				
not sequenced																				
sum of seq <sup>2</sup>	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
oomcaa <sup>1</sup>	69	70	68	68	69	69	68	69	67	67	69	39	65	38	44	70	70	34	39	68
mcaa <sup>1</sup>	Q	A	P	G	Q	G	L	E	W	M	G	G	I	I	P	-	-	I	F	G
rel. oomcaa <sup>5</sup>	99%	100%	97%	97%	99%	99%	97%	99%	96%	96%	99%	56%	93%	54%	63%	100%	100%	49%	56%	97%
pos occupied <sup>6</sup>	2	1	3	3	2	2	3	2	4	4	2	4	4	6	5	1	1	10	6	3

Table 6A: Analysis of V heavy chain subgroup 1A

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A	1	34			69											43									
B																									
C																									
D	15		1							2							70								
E									1									33							
F				1				48					3		4										
G	1						3			67															
H			1																						
I	4												1	44				1							
K	1		2	1			47		1		1							8							
L	1	1						22					2		1		3								
M															21										
N	9		59				18																		
P	1	7																							
Q	1	1				70			64																
R	2						2		1		69							1							
S		1	2		1											5			70						
T	34	26	4						3				66		65	24		27		67					
V										1		65	3							3					
W																									
X																									
Y			1	68																					
Z																									
-																									
unknown (?)																									
not sequenced																									
sum of seq <sup>2</sup>	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70					
oomcaa <sup>3</sup>	34	34	59	68	69	70	47	48	64	67	69	65	66	44	65	43	70	33	70	67					
mcaa <sup>4</sup>	T	A	N	Y	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T					
rel. oomcaa <sup>5</sup>	49%	49%	84%	97%	99%	100%	67%	69%	91%	96%	99%	93%	94%	63%	93%	61%	100%	47%	100%	96%					
pos occupied <sup>6</sup>	11	6	7	3	2	1	4	2	5	3	2	3	3	4	2	3	1	5	1	2					

Table 6A: Analysis of V heavy chain subgroup 1A

amino acid <sup>1</sup>	Framework III																
	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89
A			64			1						3			1	70	
B																	
C																	70
D						2							26	70			
E						64							44				
F																1	1
G									1								2
H				1				1									
I		1					3	1	1								2
K												3					
L					3		63			70							2
M					67										1		1
N	4							1	16								
P																	
Q				1		3											
R	3							23	1		62						
S	62		1					41	49			67			1		
T	1	69	2					3	2		4				67		
V			3				4				1					64	
W																	
X																	
Y				68													69
Z																	68
-																	
unknown (?)																	
not sequenced																	
sum of seq <sup>2</sup>	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
oomcaa <sup>3</sup>	62	69	64	68	67	64	63	41	49	70	62	67	44	70	67	70	64
mcaa <sup>4</sup>	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V
rel. oomcaa <sup>5</sup>	89%	99%	91%	97%	96%	91%	90%	59%	70%	100%	89%	96%	63%	100%	96%	100%	91%
pos occupied <sup>6</sup>	4	2	4	3	2	4	3	6	6	1	4	2	2	1	4	1	5



Table 6A: Analysis of V heavy chain subgroup 1A

amino acid <sup>1</sup>	CDR III																		
	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K
A	66	2	16		1	1	1	4	1	2	2	1	1		1	1	1	2	1
B																			
C					1	1	16	2		1	1	7	2	1					
D			16	5	3		3	5	4	3	4			1	1	14			59
E			9				2			1			1			1			
F					1	3		2		3	1	2		2	1			28	2
G		2	14	13	20	10	14	5	20	15	16	3	3	4	15	1	1	7	
H										1	1	1		1					
I				2	5	2	2		2	2	1	1			1				
K		5			2	1			1										
L		1	4	4	2	5	2	1	1		4	2		1			1	1	
M			1		2		1		1			1	1					10	
N				2	2	1	2	1	2	2	2	2			1	1	4		
P				20	3		1	3	2	2	2	4	2	1	4	1		1	1
Q				1			1		1	1	1								
R		55	1	5	7	8	1	4		2		1		16					
S		1	1	5	5	5	5	21	5	11	8	4	3		2	1		2	1
T	1	3	3	5	4	1	3	4	2	5	2		1			1	1		
V	3		3	2	4	3	3	3	4	2	2	2	1	2	1				
W				1	1	3	1	1			2		3				1	5	1
X																			
Y		1		2	3	20	5	4	9	1	2	11	20	10	6	9	10	7	1
Z																			
-				1	2	2	3	6	11	11	14	23	26	26	31	34	46	39	21
unknown (?)													1		1	1		2	3
not sequenced			2	2	2	4	4	4	4	5	5	5	5	5	5	5	5	5	5
sum of seq <sup>2</sup>	70	70	68	68	68	66	66	66	66	65	65	65	65	65	65	65	65	65	65
oomcaa <sup>3</sup>	66	55	16	20	20	20	16	21	20	15	16	23	26	26	31	34	46	39	28
mcaa <sup>4</sup>	A	R	A	P	G	Y	C	S	G	-	-	-	-	-	-	-	-	-	F
rel. oomcaa <sup>5</sup>	94%	79%	24%	29%	29%	30%	24%	32%	30%	23%	25%	35%	40%	40%	48%	52%	71%	60%	43%
pos occupied <sup>6</sup>	3	8	10	14	18	15	18	15	15	17	17	15	12	11	11	10	8	7	6

Table 6A: Analysis of V heavy chain subgroup 1A

Framework IV													
amino acid <sup>1</sup>	102	103	104	105	106	107	108	109	110	111	112	113	sum
A													670
B													
C													165
D		1	1										308
E	1	1											297
F	2												226
G			58		59	1	1						928
H				1									14
I	3								4				286
K				3		1							325
L	3			1			40	1					386
M	1						3						189
N				1									176
P	5											1	238
Q				52									494
R				1									351
S											53	51	972
T						54	11	1	51		1		736
V	15		1				1	54		54		1	699
W		59		1									243
X													
Y	34		1										542
Z													3
-	1												578
unknown (?)													8
not sequenced	5	9	9	10	11	14	14	14	15	16	16	17	406
sum of seq <sup>2</sup>	65	61	61	60	59	56	56	56	55	54	54	53	
oomcaa <sup>3</sup>	34	59	58	52	59	54	40	54	51	54	53	51	
mcaa <sup>4</sup>	Y	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	52%	97%	95%	87%	100%	96%	71%	96%	93%	100%	98%	96%	
pos occupied <sup>6</sup>	9	3	4	7	1	3	5	3	2	1	2	3	

Table 6B: Analysis of V heavy chain subgroup 1B

Framework I																				
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A									32							34				
B																				
C																				
D																				
E		1			5	1				35										
F																				
G								27								35				
H			1											1						
I																				1
K		3	1									34	33						33	
L			3	26	1															
M				1	1															
N																				
P									1					33			1			
Q	21		20			26														
R	1											1	2							
S							27										1	34		
T									1					1					2	
V	3	21			20						35							35		34
W																				
X																				
Y																				
Z																				
-																				
unknown (?)																				
not sequenced	15	15	15	13	13	13	13	13	6	5	5	5	5	5	5	5	5	5	5	5
sum of seq <sup>2</sup>	25	25	25	27	27	27	27	27	34	35	35	35	35	35	35	35	35	35	35	35
oomcaa <sup>3</sup>	21	21	20	26	20	26	27	27	32	35	35	34	33	33	35	34	34	35	33	34
mcaa <sup>4</sup>	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V
rel. oomcaa <sup>5</sup>	84%	84%	80%	96%	74%	96%	100%	100%	94%	100%	100%	97%	94%	94%	100%	97%	97%	100%	94%	97%
pos occupied <sup>6</sup>	3	3	4	2	4	2	1	1	3	1	1	2	2	3	1	2	2	1	2	2

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Table 6B: Analysis of V heavy chain subgroup 1B

											CDRI																	
amino acid <sup>1</sup>	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38								
A				30							2				6													
B																												
C		35																										
D											1				5		1			1								
E			3								1																	
F							2	39						2	2													
G				1	40					1	14					1				1								
H														3	1		34											
I								1		1							9											
K			28																									
L									1		1					5			2									
M																23												
N							1			1	3						1	3										
P																1												
Q			2								1					1		1		1								
R			2					2							1					37								
S	35			40				5		2	15				2	1												
T				3				32		34						1												
V				1			1			1	1					2	2			38								
W																		40										
X																												
Y							36				1			32	19		1											
Z																												
-												40	40															
unknown (?)																												
not sequenced	5	5	5	5																								
sum of seq <sup>2</sup>	35	35	35	35	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40								
oomcaa <sup>3</sup>	35	35	28	30	40	40	36	32	39	34	15	40	40	32	19	23	34	40	38	37								
mcaa <sup>4</sup>	S	C	K	A	S	G	Y	T	F	T	S	-	-	Y	Y	M	H	W	V	R								
rel. oomcaa <sup>5</sup>	100%	100%	80%	86%	100%	100%	90%	80%	98%	85%	38%	100%	100%	80%	48%	58%	85%	100%	95%	93%								
pos occupied <sup>6</sup>	1	1	4	4	1	1	4	4	2	6	10	1	1	5	11	5	5	1	2	4								

Table 6B: Analysis of V heavy chain subgroup 1B

	Framework II																			
amino acid <sup>1</sup>	39	40	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55
A		39				1					1				7			1		
B																				
C																				
D														1					1	
E				1				39										1	1	
F							2						1					1		
G				39		28					39	1			1			9	1	39
H																		2		
I										3			34							
K					1														1	
L			1				37						1							
M									37		2	4								
N														35				20	12	1
P		1	34				1								31					
Q	39				39			1												
R	1					10						4						3	1	
S			1			1								2				1	20	
T			4											1					3	
V														1	1					
W									40			33								
X																				
Y																		2		
Z																				
-																40	40			
unknown (?)																				
not sequenced																				
sum of seq <sup>2</sup>	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
oomcaa <sup>3</sup>	39	39	34	39	39	28	37	39	40	37	39	33	34	35	31	40	40	20	20	39
mcaa <sup>4</sup>	Q	A	P	G	Q	G	L	E	W	M	G	W	I	N	P	-	-	N	S	G
rel. oomcaa <sup>5</sup>	98%	98%	85%	98%	98%	70%	93%	98%	100%	93%	98%	83%	85%	88%	78%	100%	100%	50%	50%	98%
pos occupied <sup>6</sup>	2	2	4	2	2	4	3	2	1	2	2	4	4	5	4	1	1	9	8	2

Table 6B: Analysis of V heavy chain subgroup 1B

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A	1	2			27	2				1		1				2				12					
B																									
C																									
D	1									4							35								
E	2		2			1				1						1									
F				4				39							3										
G	15		6		1					34															
H			1	1													1								
I		1	1									1	1	13						22					
K	2	2	8				36		1							1									
L						1		1							1										
M														23				1		1					
N	17		18				1										4								
P																			3						
Q						36			37																
R			2				1		2	37						34		1							
S	1			2	11		1									1			37						
T		35	2		1	1							39		40	1		38		5					
V	1											38													
W										3															
X																									
Y				33																					
Z																									
-																									
unknown (?)																									
not sequenced																									
sum of seq <sup>1</sup>	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40					
oomcaa <sup>1</sup>	17	35	18	33	27	36	36	39	37	34	37	38	39	23	40	34	35	38	37	22					
mcaa <sup>4</sup>	N	T	N	Y	A	Q	K	F	Q	G	R	V	T	M	T	R	D	T	S	I					
rel. oomcaa <sup>5</sup>	43%	88%	45%	83%	68%	90%	90%	98%	93%	85%	93%	95%	98%	58%	100%	85%	88%	95%	93%	55%					
pos occupied <sup>6</sup>	8	4	8	4	4	4	5	2	3	4	2	3	2	4	1	6	3	3	2	4					

Table 6B: Analysis of V heavy chain subgroup 1B

amino acid <sup>1</sup>	Framework III																			
	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92
A			35									1	2			40				
B																				
C																				37
D	1					4							19	40			1			
E						35							19							
F			1									2							2	1
G						1		1	2											
H																				
I		1															1			
K											1									
L					2	39				39							2			1
M					37		1										2			
N	7							1	2											
P												1							1	
Q																				
R	4							2	16		37									
S	27			1				35	20		1	36						1	1	
T	1	39						1			1				40					
V			4		1					1							33			
W																				
X																				
Y				39														38	35	
Z																				
-																				
unknown (?)																				
not sequenced																	1	1	1	1
sum of seq <sup>2</sup>	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	39	39	39	39
oomcaa <sup>3</sup>	27	39	35	39	37	35	39	35	20	39	37	36	19	40	40	40	33	38	35	37
mcaa <sup>4</sup>	S	T	A	Y	M	E	L	S	S	L	R	S	D	D	T	A	V	Y	Y	C
rel. oomcaa <sup>5</sup>	68%	98%	88%	98%	93%	88%	98%	88%	50%	98%	93%	90%	48%	100%	100%	100%	85%	97%	90%	95%
pos occupied <sup>6</sup>	5	2	3	2	3	3	2	5	4	2	4	4	3	1	1	1	5	2	4	3

Table 6B: Analysis of V heavy chain subgroup 1B

amino acid <sup>1</sup>	CDR III																			
	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K	101
A	37	1	6		1	1		2	3	1	3		1					5		
B																				
C		1				3				2	1									
D			7		5	2	3	1	5	4		1		2	2	1	2			27
E			2		1			1	1		2		1		1					
F				1	1	3			2	1	1	1	1					2	15	
G		1	7	7	5	5	9	4	7	1	3		2	2	1		1	3		1
H			1				2			1	1									
I		1		1	1	3	1	1	1	1	1	1							1	
K		1			1				1	1		1		1			1			
L			2	4	4	4	3			1	2	1	1	2		1			2	
M				2		1	1								1				4	
N					1			1		1	1	1			3		1		1	
P				6	4				1	1		3	2				1			
Q					1							1	2	1						
R	1	31		5	1	1	3					1		1				1		
S		1	3	3	1	4	3	6	3	2	2	1		1						
T		2	1	1	2	2	1	5	1	1	1		1			1		1		
V	1		7	1	1		1	3	1	2		1			1	2	1		1	
W			1		1		2	2		1	1					1		4		
X																				
Y				5	5	4	2	3		4	3	3	2	1	2	5	6	2		
Z																				
-				1	1	4	6	8	10	11	14	20	23	25	25	25	23	18	11	6
unknown (?)																			3	
not sequenced	1	1	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
sum of seq <sup>2</sup>	39	39	37	37	37	37	37	37	36	36	36	36	36	36	36	36	36	36	36	36
oomcaa <sup>3</sup>	37	31	7	7	5	5	9	8	10	11	14	20	23	25	25	25	23	18	15	27
mcaa <sup>4</sup>	A	R	D	G	D	G	G	-	-	-	-	-	-	-	-	-	-	-	F	D
rel. oomcaa <sup>5</sup>	95%	79%	19%	19%	14%	14%	24%	22%	28%	31%	39%	56%	64%	69%	69%	69%	64%	50%	42%	75%
pos occupied <sup>6</sup>	3	8	10	12	18	13	13	12	12	17	14	13	10	9	8	7	8	8	5	5



Table 6B: Analysis of V heavy chain subgroup 1B

amino acid <sup>1</sup>	Framework IV												sum
	102	103	104	105	106	107	108	109	110	111	112	113	
A													340
B													
C													79
D	2												179
E				1									159
F	1												130
G			27		26					1			450
H	1												51
I	7								3				113
K				2									194
L							12			1			204
M							2						144
N	1												138
P	1			1									128
Q				23									253
R							1						247
S	3								1		18	18	432
T						21	6		16		1		390
V	6							21		18			342
W		29											158
X													
Y	11												294
Z													
-	3												394
unknown (?)													3
not sequenced	4	11	13	13	14	19	19	19	20	20	21	22	458
sum of seq <sup>2</sup>	36	29	27	27	26	21	21	21	20	20	19	18	
oomcaa <sup>3</sup>	11	29	27	23	26	21	12	21	16	18	18	18	
mcaa <sup>4</sup>	Y	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	31%	100%	100%	85%	100%	100%	57%	100%	80%	90%	95%	100%	
pos occupied <sup>6</sup>	10	1	1	4	1	1	4	1	3	3	2	1	

Table 6C: Analysis of V heavy chain subgroup 2

Framework I																				
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A										3										
B																				
C																				
D																				
E	1					6										2				
F																				
G								6												
H																				
I		1																		
K					3								6		1					
L				6						6								6		6
M																				
N							1													
P							1		6					6			1			
Q	2															4				
R					2															
S							4													
T			6		1					2					5		5		6	
V		5								1		6								
W																				
X																				
Y																				
Z	3																			
-																				
unknown (?)																				
not sequenced	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
sum of seq <sup>2</sup>	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
oomcaa <sup>3</sup>	3	5	6	6	3	6	4	6	6	3	6	6	6	6	5	4	5	6	6	6
mcaa <sup>4</sup>	Z	V	T	L	K	E	S	G	P	A	L	V	K	P	T	Q	T	L	T	L
rel. oomcaa <sup>5</sup>	50%	83%	100%	100%	50%	100%	67%	100%	100%	50%	100%	100%	100%	100%	83%	67%	83%	100%	100%	100%
pos occupied <sup>6</sup>	3	2	1	1	3	1	3	1	1	3	1	1	1	1	2	2	2	1	1	1

Table 6C: Analysis of V heavy chain subgroup 2

											CDRI									
amino acid <sup>1</sup>	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38
A								1				1			1					
B																				
C		7													2					
D												1								
E																				
F				3			6		1											
G						7							4		3		3			
H																				
I													1						7	
K																				
L				2			1		6											
M															5					
N											2									
P																				
Q																				
R														2		1				7
S			1		6			6		6	2	4					4			
T	6		6							1	3	1								
V				2											2		7			
W																		7		
X																				
Y					1															
Z																				
-																				
unknown (?)																				
not sequenced	1																			
sum of seq <sup>2</sup>	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
oomcaa <sup>3</sup>	6	7	6	3	6	7	6	6	6	6	3	4	4	5	3	7	4	7	7	7
mcaa <sup>4</sup>	T	C	T	F	S	G	F	S	L	S	T	S	G	M	G	V	S	W	I	R
rel. oomcaa <sup>5</sup>	100%	100%	86%	43%	86%	100%	86%	86%	86%	86%	43%	57%	57%	71%	43%	100%	57%	100%	100%	100%
pos occupied <sup>6</sup>	1	1	2	3	2	1	2	2	2	2	3	4	3	2	4	1	2	1	1	1

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Table 6C: Analysis of V heavy chain subgroup 2

amino acid <sup>1</sup>	Framework II																	A	B	C	53	54	55
	39	40	41	42	43	44	45	46	47	48	49	50	51	52									
A						6					7												
B																							
C																							
D														2							3	6	
E								7															
F														2									
G		1		7		1																	
H												2										1	
I													6										
K					6																		
L							7			7		2	1	1									
M																							
N																					3		
P		5	7																				
Q	6																						
R	1				1							2											
S		1																		2			
T																							
V																							
W								7			1									4			
X														1						1	1		
Y														1	1								
Z																							
-																							
unknown (?)														6	7	7							
not sequenced																							
sum of seq <sup>2</sup>	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
oomcaa <sup>3</sup>	6	5	7	7	6	6	7	7	7	7	7	2	6	2	6	7	7	4	3	6			
mcaa <sup>4</sup>	Q	P	P	G	K	A	L	E	W	L	A	H	I	D	-	-	-	W	D	D			
rel. oomcaa <sup>5</sup>	86%	71%	100%	100%	86%	86%	100%	100%	100%	100%	100%	29%	86%	29%	86%	100%	100%	57%	43%	86%			
pos occupied <sup>6</sup>	2	3	1	1	2	2	1	1	1	1	1	4	2	5	2	1	1	3	3	2			

Table 6C: Analysis of V heavy chain subgroup 2

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A																									
B																									
C																									
D	5																6	1							
E	1								1																
F		1		1																					
G																									
H				1																					
I														6											
K	1	6							4							6				6					
L								7				7													
M																									
N																	1								
P						2																			
Q																									
R			2			1			2	7						1				1					
S			2		6		7			4			1		5				7						
T						4				3			6		2			6							
V														1											
W				1																					
X					1																				
Y			3	4																					
Z																									
-																									
unknown (?)																									
not sequenced																									
sum of seq <sup>2</sup>	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7					
oomcaa <sup>3</sup>	5	6	3	4	6	4	7	7	4	4	7	7	6	6	5	6	6	6	7	6					
mcaa <sup>4</sup>	D	K	Y	Y	S	T	S	L	K	S	R	L	T	I	S	K	D	T	S	K					
rel. oomcaa <sup>5</sup>	71%	86%	43%	57%	86%	57%	100%	100%	57%	57%	100%	100%	86%	86%	71%	86%	86%	86%	100%	86%					
pos occupied <sup>6</sup>	3	2	3	4	2	3	1	1	3	2	1	1	2	2	2	2	2	2	1	2					

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Table 6C: Analysis of V heavy chain subgroup 2

Framework III																						
amino acid <sup>1</sup>	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92		
A													1			5						
B																						
C																						
D											6			7							7	
E																						
F					1																	
G																						
H																2						
I						2		1														
K																						
L					6																	
M							7			5												
N	5								6		1											
P												7										
Q		7																				
R																						
S	2																					
T					5		5								7		7					
V			7	7						1			6									
W																						
X																						
Y																						
Z																		7	7			
-								1	1	1												
unknown (?)																						
not sequenced																						
sum of seq <sup>2</sup>	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
oomcaa <sup>3</sup>	5	7	7	7	6	5	7	5	6	5	6	7	6	7	7	5	7	7	7	7	7	
mcaa <sup>4</sup>	N	Q	V	V	L	T	M	T	N	M	D	P	V	D	T	A	T	Y	Y	C		
rel. oomcaa <sup>5</sup>	71%	100%	100%	100%	86%	71%	100%	71%	86%	71%	86%	100%	86%	100%	100%	71%	100%	100%	100%	100%	100%	
pos occupied <sup>6</sup>	2	1	1	1	2	2	1	3	2	3	2	1	2	1	1	2	1	1	1	1	1	

Table 6C: Analysis of V heavy chain subgroup 2

	CDR III																			
amino acid <sup>1</sup>	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K	101
A	5							1	2	1										
B																				
C																				
D																				6
E								2			1									
F																			3	
G						1	1		1	2	1	1	1	1						
H		1		1																
I			3			2														
K							1													
L								1		1									1	
M								1											2	
N				1	2												1			
P				1	1		1		1											
Q			1																	
R		6	1			1			1											
S				1		1	1													
T				1			1		1											
V	2		1	1	1		1	1			1									
W						1										1		1		
X																				
Y					2						1	2	1	1	1			2		
Z																				
-										2	2	3	4	4	4	6	5	3		
unknown (?)																				
not sequenced			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
sum of seq <sup>2</sup>	7	7	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
oomcaa <sup>3</sup>	5	6	3	1	2	2	1	2	2	2	2	3	4	4	4	6	5	3	3	6
mcaa <sup>4</sup>	A	R	I	H	N	I	G	E	A	-	-	-	-	-	-	-	-	-	F	D
rel. oomcaa <sup>5</sup>	71%	86%	50%	17%	33%	33%	17%	33%	33%	33%	33%	50%	67%	67%	67%	100%	83%	50%	50%	100%
pos occupied <sup>6</sup>	2	2	4	6	4	5	6	5	5	4	5	3	3	3	3	1	2	3	3	1

Table 6C: Analysis of V heavy chain subgroup 2

Framework IV													
amino acid <sup>1</sup>	102	103	104	105	106	107	108	109	110	111	112	113	sum
A									1				35
B													
C													16
D													43
E													21
F													18
G			6		6								55
H													6
I													29
K				1			1						42
L	1						3						78
M													20
N													23
P	1						1						41
Q				3									23
R				2									41
S											6	3	82
T						6	1		5				102
V	3							6		6			68
W		6											29
X													4
Y	1												35
Z													3
-													56
unknown (?)													
not sequenced	1	1	1	1	1	1	1	1	1	1	1	4	54
sum of seq <sup>2</sup>	6	6	6	6	6	6	6	6	6	6	6	3	
oomcaa <sup>3</sup>	3	6	6	3	6	6	3	6	5	6	6	3	
mcaa <sup>4</sup>	V	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	50%	100%	100%	50%	100%	100%	50%	100%	83%	100%	100%	100%	
pos occupied <sup>6</sup>	4	1	1	3	1	1	4	1	2	1	1	1	

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Table 6D: Analysis of V heavy chain subgroup 3

	Frame														
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A					1		1			12		1		3	1
B			1			1							1		
C															
D	1					1				16					
E	110		9		15	166			9				8		2
F											4				
G								181	193	174		1			202
H			5										4		
I												9			
K		5	3										26		
L		1	5	176	43						140			1	
M		12		1											
N										1					
P													1	194	
Q	41		138	1	3	12							162		
R			6										4		
S							178			2				8	
T							1								
V	5	147		1	118						62	195			
W															1
X															
Y															
Z	8														
-															
unknown (?)															
not sequenced	47	47	45	33	32	32	32	31	10	7	6	6	6	6	6
sum of seq <sup>2</sup>	165	165	167	179	180	180	180	181	202	205	206	206	206	206	206
oomcaa <sup>3</sup>	110	147	138	176	118	166	178	181	193	174	140	195	162	194	202
mcaa <sup>4</sup>	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G
rel. oomcaa <sup>5</sup>	67%	89%	83%	98%	66%	92%	99%	100%	96%	85%	68%	95%	79%	94%	98%
pos occupied <sup>6</sup>	5	4	7	4	5	4	3	1	2	5	3	4	7	4	4

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Table 6D: Analysis of V heavy chain subgroup 3

work I															
amino acid <sup>1</sup>	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A								183	192		1				
B															
C						1	209								
D															7
E	8							8			3		1		
F		1	1			1						201		201	
G	134								2		207				3
H															1
I								2				3	17	1	
K				15											4
L			205		201							6		3	
M			1										1		
N													10		10
P								1					2		
Q			1												
R	62			191											11
S		206				207		4	2	209			15		174
T	4	1		2				4	4			1	163		
V					8			7	9				1	6	
W															
X															
Y															
Z															
-															
unknown (?)															
not sequenced	4	4	4	4	3	3	3	3	3	3	1	1	2	1	2
sum of seq <sup>2</sup>	208	208	208	208	209	209	209	209	209	209	211	211	210	211	210
oomcaa <sup>3</sup>	134	206	205	191	201	207	209	183	192	209	207	201	163	201	174
mcaa <sup>4</sup>	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S
rel. oomcaa <sup>5</sup>	64%	99%	99%	92%	96%	99%	100%	88%	92%	100%	98%	95%	78%	95%	83%
pos occupied <sup>6</sup>	4	3	4	3	2	3	1	7	5	1	3	4	8	4	7

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Table 6D: Analysis of V heavy chain subgroup 3

amino acid <sup>a</sup>	CDRI										Frame				
	31	A	B	32	33	34	35	36	37	38	39	40	41	42	43
A	1			17	80		1			1		187		1	
B															
C												1		1	
D	26			3	7		2								
E	1				10									1	1
F				5											
G	13				31		1					2		209	
H				4			88								
I	1			1		15			12						
K	7										1				202
L	3					3			2	3	1	2	1		
M						193									
N	35			8	3		34								
P				1			1					4	191		
Q											209		1		1
R	7									207		7			8
S	103			17	8		72					3	14		
T	9				15		10					4	5		
V	2				7	1			197			2			
W					30			212							
X	1														
Y	1			154	19		3								
Z															
-		210	210												
unknown (?)															
not sequenced <sup>b</sup>	2			2	2				1	1	1				
sum of seq <sup>c</sup>	210	210	210	210	210	212	212	212	211	211	211	212	212	212	212
oomcaa <sup>d</sup>	103	210	210	154	80	193	88	212	197	207	209	187	191	209	202
mcaa <sup>e</sup>	S	-	-	Y	A	M	H	W	V	R	Q	A	P	G	K
rel. oomcaa <sup>f</sup>	49%	100%	100%	73%	38%	91%	42%	100%	93%	98%	99%	88%	90%	99%	95%
pos occupied <sup>g</sup>	14	1	1	9	10	4	9	1	3	3	3	9	5	4	4

Table 6D: Analysis of V heavy chain subgroup 3

work II															
amino acid <sup>1</sup>	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55
A	1					77	42		1	2		14		7	
B			3							1					
C													1		
D			1							7			94	8	3
E			198						3	2	1		2		1
F							7	1	2	1				1	8
G	207					33	11		10	46			4	163	85
H							6			1					
I					3		3	191		1					1
K								1	37	2	30		3	1	
L		211			5		12	1							
M							1	1							
N							13		7	9	2		13	11	1
P		1								1			1		
Q			7				7			10					
R	1						24	1	17	5	1		2		16
S	3			1		102	11	9	118	43		1	74	17	82
T							3	5	4	2		13	12	3	3
V			3		204		49	2		1		6			
W				210			1		8	6					
X													4		3
Y				1			22		5	58					8
Z															
-										14	178	178	2	1	1
unknown (?)															
not sequenced															
sum of seq <sup>2</sup>	212	212	212	212	212	212	212	212	212	212	212	212	212	212	212
oomcaa <sup>3</sup>	207	211	198	210	204	102	49	191	118	58	178	178	94	163	85
mcaa <sup>4</sup>	G	L	E	W	V	S	V	I	S	Y	-	-	D	G	G
rel. oomcaa <sup>5</sup>	98%	100%	93%	99%	96%	48%	23%	90%	56%	27%	84%	84%	44%	77%	40%
pos occupied <sup>6</sup>	4	2	5	3	3	3	15	9	11	19	5	5	12	9	12

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Table 6D: Analysis of V heavy chain subgroup 3

	CDR II															
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	
A	9	1	2		174	33							1			
B	1	2														
C																
D	11		17			160										
E	8	3	2			1			2							
F	1		3	2								207				
G	5	1	5		4	5				212	1					
H	1		4													
I	3	37	2					8					14	208		
K	1	61							199		8					
L	1	1	1		1							1		1		
M	8		2		1											
N	51		4			2			2							
P	1	1			6	8	18		1							
Q	3	2							2		2					
R	5	4			5				6		201					
S	48		11		4		193					2	7		211	
T	42	97	5		7								189		1	
V		2			10	2		204				1		3		
W			2													
X	4		1			1										
Y	9		151	210			1					1	1			
Z																
-																
unknown (?)																
not sequenced																
sum of seq <sup>2</sup>	212	212	212	212	212	212	212	212	212	212	212	212	212	212	212	
oomcaa <sup>3</sup>	51	97	151	210	174	160	193	204	199	212	201	207	189	208	211	
mcaa <sup>4</sup>	N	T	Y	Y	A	D	S	V	K	G	R	F	T	I	S	
rel. oomcaa <sup>5</sup>	24%	46%	71%	99%	82%	75%	91%	96%	94%	100%	95%	98%	89%	98%	100%	
pos occupied <sup>6</sup>	19	12	15	2	9	8	3	2	6	1	4	5	5	3	2	

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Table 6D: Analysis of V heavy chain subgroup 3

amino acid <sup>1</sup>	Framework III														
	71	72	73	74	75	76	77	78	79	80	81	82	A	B	C
A				57			1	8						1	
B											2				
C															
D		199	38		2	2			1				10		
E		6			4						5				
F									13						
G													1	4	
H						1			1		2		2		
I			1				2	2				3	1	1	
K					186	6							3		
L								188		209		3	1		212
M	1				2		10	3		2		205			
N		5	170		2	188					3		181	10	
P							1								
Q					7						199				
R	211				1	1							2	8	
S				153	8	10	56		3				6	186	
T							142				1		4	2	
V				1				11		1		1			
W															
X		2	2			4							1		
Y									194						
Z															
-															
unknown (?)															
not sequenced <sup>2</sup>			1	1											
sum of seq <sup>3</sup>	212	212	211	211	212	212	212	212	212	212	212	212	212	212	212
oomcaa <sup>3</sup>	211	199	170	153	186	188	142	188	194	209	199	205	181	186	212
mcaa <sup>4</sup>	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L
rel. oomcaa <sup>5</sup>	100%	94%	81%	73%	88%	89%	67%	89%	92%	99%	94%	97%	85%	88%	100%
pos occupied <sup>6</sup>	2	4	4	3	8	7	6	5	5	3	6	4	11	7	1

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Table 6D: Analysis of V heavy chain subgroup 3

amino acid <sup>1</sup>	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97
A		149	1		1	207					173	2	15	9	11
B															
C									1	210		5	2		1
D		5	15	209								2	54	7	6
E	1		190										11	2	11
F							1		15			1		9	6
G	1	1	6			4	1				2	8	34	26	35
H		1							1					3	11
I		8					2						4	15	10
K	30											60	4	3	5
L							18					1	6	11	7
M					2		1							6	1
N		1		1								2	20	4	3
P		9									1	3	4	29	10
Q				1								5	3	9	2
R	177											103	9	30	19
S		1			1							3	9	8	11
T	3	28			207		1				25	15	7	6	20
V		9					187				10	1	7	7	15
W										1			3	4	3
X				1											
Y								211	194				12	9	8
Z															
-													1	3	4
unknown (?)															
not sequenced					1	1	1	1	1	1	1	1	7	12	13
sum of seq <sup>2</sup>	212	212	212	212	211	211	211	211	211	211	211	211	205	200	199
oomcaa <sup>3</sup>	177	149	190	209	207	207	187	211	194	210	173	103	54	30	35
mcaa <sup>4</sup>	R	A	E	D	T	A	V	Y	Y	C	A	R	D	R	G
rel. oomcaa <sup>5</sup>	83%	70%	90%	99%	98%	98%	89%	100%	92%	100%	82%	49%	26%	15%	18%
pos occupied <sup>6</sup>	5	10	4	4	4	2	7	1	4	2	5	14	18	20	21

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Table 6D: Analysis of V heavy chain subgroup 3

amino acid <sup>1</sup>	CDR III															
	98	99	100	A	B	C	D	E	F	G	H	I	J	K	101	
A	7	13	7	9	6	2	3	5	5		9		13		2	
B																
C	13	5		1	2	11	3		2					1		
D	11	7	10	4	2	3	10	3	3	1		3	2		146	
E	6	3	1	13		1	1								1	
F	3	5	4	5	5	6	3	5	7	2		1	1	65	1	
G	34	17	35	17	14	23	10	5	1	5	3	2	32		6	
H	3	4	3	2	9	2		1	3	1	2	8	1			
I	6	11	4	4	3	1	3	10	3	3	2		1	2		
K	2	11			3	1										
L	26	13	4	12	8	2	6	3	10	3				2	1	
M		1	2								1			32		
N	4	6	4	3	2	2	6				2	5			2	
P	6	5	5	6	9	8	2	3	2	1		3		9		
Q	4		1	1	1	1	1					1				
R	4	10	9	7	5	5	2	3	1		1		2		4	
S	16	28	27	25	24	8	11	9	3		2	3	1	1	1	
T	6	12	9	17	17	1	2	5	1	9	3	1				
V	13	7	15	4	3	6	2	12		1	1	1	1			
W	6	5	6	7	2	4				1		6	10			
X				1											1	
Y	16	14	17	5	8	18	20	13	20	25	28	32	28			
Z																
-	12	21	35	54	73	87	102	110	126	135	134	120	91	71	21	
unknown (?)							3	2	1	1			3	2		
not sequenced	14	14	14	14	15	19	21	22	23	23	23	25	25	26	25	
sum of seq <sup>2</sup>	198	198	198	197	196	192	190	189	188	188	188	186	186	185	186	
oomcaa <sup>3</sup>	34	28	35	54	73	87	102	110	126	135	134	120	91	71	146	
mcaa <sup>4</sup>	G	S	G	-	-	-	-	-	-	-	-	-	-	-	D	
rel. oomcaa <sup>5</sup>	17%	14%	18%	27%	37%	45%	54%	58%	67%	72%	71%	65%	49%	38%	78%	
pos occupied <sup>6</sup>	20	20	19	20	19	20	17	14	14	12	12	13	12	8	11	

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Table 6D: Analysis of V heavy chain subgroup 3

amino acid <sup>1</sup>	Framework IV												sum
	102	103	104	105	106	107	108	109	110	111	112	113	
A	1		1			2							1767
B				1									13
C													470
D	2												1121
E					1								832
F	2												807
G			140		130		1						2743
H	4												179
I	15								1	1			651
K				13									933
L	10			1			91					2	1881
M							6						496
N	1					1							844
P	17					1	1						568
Q				111									949
R				8									1413
S	7	1									118	110	3009
T						123	27		122			1	1426
V	34		1			1		125		119			1851
W		158											686
X													26
Y	82												1598
Z													8
-	9	2	2	2	2	2	2	2	2	2	1	1	2023
unknown (?)													12
not sequenced	27	50	67	75	78	81	83	84	86	89	92	97	1650
sum of seq <sup>2</sup>	184	161	144	136	133	130	128	127	125	122	119	114	
oomcaa <sup>3</sup>	82	158	140	111	130	123	91	125	122	119	118	110	
mcaa <sup>4</sup>	Y	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	45%	98%	97%	82%	98%	95%	71%	98%	98%	98%	99%	96%	
pos occupied <sup>6</sup>	12	3	4	6	3	6	6	2	3	3	2	4	

Table 6E: Analysis of V heavy chain subgroup 4

Framework I																				
amino acid <sup>1</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A									19					1			1		1	
B																				
C																				
D																				
E					32											44				
F																				
G								54	1	53						2				
H			4		2															
I																				
K												1	54						1	
L		7		54							53	19		1				53		50
M																				
N																				
P									33					51	1					2
Q	52		50		51	20										7				
R	1																			
S						33									52				52	
T									1								52			
V		47				1						34								1
W						20														
X																				
Y																				
Z	1																			
-																				
unknown (?)																				
not sequenced	3	3	3	3	4	4	4	3	3	4	4	3	3	4	4	4	4	4	3	4
sum of seq <sup>2</sup>	54	54	54	54	53	53	53	54	54	53	53	54	54	53	53	53	53	53	54	53
oomcaa <sup>3</sup>	52	47	50	54	51	32	33	54	33	53	53	34	54	51	52	44	52	53	52	50
mcaa <sup>4</sup>	Q	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L
rel. oomcaa <sup>5</sup>	96%	87%	93%	100%	96%	60%	62%	100%	61%	100%	100%	63%	100%	96%	98%	83%	98%	100%	96%	94%
pos occupied <sup>6</sup>	3	2	2	1	2	3	2	1	4	1	1	3	1	3	2	3	2	1	3	3

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Table 6E: Analysis of V heavy chain subgroup 4

											CDRI																
amino acid <sup>1</sup>	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38							
A			22											1													
B																											
C		53													1												
D			1								4	1	1	1			1										
E																											
F					1				22					1	1				1								
G						53	53				21	3	4				8										
H							1							2													
I			1					1	32										51								
K																											
L																			1								
M																											
N										1	1		2	2			1										
P								3																			
Q											1																
R						1				3	2		1							57							
S			2		35			51	1	52	25	5	9	1			44		1								
T	53		29								2	1					3										
V				55		1			1										3								
W												1				2	56		57								
X																											
Y					19		1								48	52											
Z																											
-												45	39														
unknown (?)																											
not sequenced	4	4	2	2	2	2	2	2	1	1	1			1	1	1											
sum of seq <sup>2</sup>	53	53	55	55	55	55	55	55	56	56	56	56	56	56	56	56	57	57	57	57							
oomcaa <sup>3</sup>	53	53	29	55	35	53	53	51	32	52	25	45	39	48	52	56	44	57	51	57							
mcaa <sup>4</sup>	T	C	T	V	S	G	G	S	I	S	S	-	-	Y	Y	W	S	W	I	R							
rel. oomcaa <sup>5</sup>	100%	100%	53%	100%	64%	96%	96%	93%	57%	93%	45%	80%	70%	86%	93%	100%	77%	100%	89%	100%							
pos occupied <sup>6</sup>	1	1	5	1	3	3	3	3	4	3	7	6	6	7	4	1	5	1	5	1							

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Table 6E: Analysis of V heavy chain subgroup 4

amino acid <sup>1</sup>	Framework II																	A	B	C	53	54	55
	39	40	41	42	43	44	45	46	47	48	49	50	51	52									
A			8	1							1												
B																							
C																							
D														1							1		
E				1				56			22												
F											1		1										
G				55		55					56	1									1		57
H		2																			24		
I										54		1	54										
K					54																		
L		1					55			2													
M																							
N														21									
P		50	49				2																
Q	56							1				1											
R					3	2						9		1									
S		3										7		1									
T	1	1																					
V										1				3							8	5	
W								56															
X																							
Y									1			15		32							23		
Z																							
-																							
unknown (?)															57	57	57						
not sequenced																							
sum of seq <sup>2</sup>	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57
oomcaa <sup>1</sup>	56	50	49	55	54	55	55	56	56	54	56	22	54	32	57	57	57	57	24	52	57		
mcaa <sup>1</sup>	Q	P	P	G	K	G	L	E	W	I	G	E	I	Y	-	-	-	-	H	S	G		
rel. oomcaa <sup>5</sup>	98%	88%	86%	96%	95%	96%	96%	98%	98%	95%	98%	39%	95%	56%	100%	100%	100%	100%	42%	91%	100%		
pos occupied <sup>6</sup>	2	5	2	3	2	2	2	2	2	3	2	8	2	6	1	1	1	1	5	2	1		

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Table 6E: Analysis of V heavy chain subgroup 4

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A		1									1		1			1				1					
B																									
C																									
D			2									1					55								
E																	1								
F				3														1							
G	1									1															
H			2																						
I	1	1										1	1	48		3									
K					1				53									1		51					
L						1		55				1				3				1					
M														7				2							
N	2		40		53								2							1					
P						54		1																	
Q																	1								
R	2								3		56									2					
S	49		1		2		56			56			1		56			1	57						
T	1	54	1			1			1				51		1			52							
V	1	1										53		2		50				1					
W																									
X																									
Y			11	54																					
Z																									
-																									
unknown (?)																									
not sequenced					1	1	1	1				1	1												
sum of seq <sup>2</sup>	57	57	57	57	56	56	56	56	57	57	57	56	56	57	57	57	57	57	57	57					
oomcaa <sup>3</sup>	49	54	40	54	53	54	56	55	53	56	56	53	51	48	56	50	55	52	57	51					
mcaa <sup>4</sup>	S	T	N	Y	N	P	S	L	K	S	R	V	T	I	S	V	D	T	S	K					
rel. oomcaa <sup>5</sup>	86%	95%	70%	95%	95%	96%	100%	98%	93%	98%	98%	95%	91%	84%	98%	88%	96%	91%	100%	89%					
pos occupied <sup>6</sup>	7	4	6	2	3	3	1	2	3	2	2	4	5	3	2	4	3	5	1	6					

Table 6E: Analysis of V heavy chain subgroup 4

amino acid <sup>1</sup>	Framework III																			
	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92
A												55	57			57				
B																				
C																				57
D					1									57						
E						1														
F			54						1											
G								1												
H																				
I			1					1			3									
K	3					46		2												
L		3	1		55		53			2								1		
M						1	1			1							1			
N	54					3		3	1											
P																				
Q		54			1	1														
R						2		2				1								
S			1	57		2	1	44	55		1				2				1	
T						1		4			53				55					
V							2			54		1					55			
W																				
X																				
Y																		57	56	
Z																				
-																				
unknown (?)																				
not sequenced																				
sum of seq <sup>2</sup>	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57
oomcaa <sup>3</sup>	54	54	54	57	55	46	53	44	55	54	53	55	57	57	55	57	55	57	56	57
mcaa <sup>4</sup>	N	Q	F	S	L	K	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C
rel. oomcaa <sup>5</sup>	95%	95%	95%	100%	96%	81%	93%	77%	96%	95%	93%	96%	100%	100%	96%	100%	96%	100%	98%	100%
pos occupied <sup>6</sup>	2	2	4	1	3	8	4	7	3	3	3	3	1	1	2	1	3	1	2	1

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Table 6E: Analysis of V heavy chain subgroup 4

amino acid <sup>1</sup>	CDR III																		
	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	K	101
A	56		3	3	3	2	5	4	2	2	4		2	1		1	1	12	
B																			
C					1				1										
D			6		5	5	5	4	3	2	4	3	1		1	2	1		41
E			6	1	1	2	1		1	3	1	2	1						
F				4	1	1		2	3	2	2		1	1				31	
G			25	9	10	8	10	11	4	7	7	6	1	1	1	2	1	9	
H			1				1						1			1			2
I				1		2	4	1	3	2	3		1					1	
K			2	1						2	2			1					
L			2	6	7	3	5	3	2	4	1	5	3	3		1			
M				1	4		3	1		2	1							9	
N				3					2	1	1	5	1	1			2		
P				4	5	3	1	1	2	1	1	1	2	3	1	2	1		
Q					1	1		1			1	1			3				1
R		54	4	12	2	5	5	3	2	3	1	2			2	1			
S		1	1	4	8	8	1	2	5	7	4	2	1	1	1				
T		1	1	2	1	3	4	4	3	3			1	1	1				
V	1	1	4	2	2	5	4	4	7	3	1	2	1						
W			1	2	1	2	2	4	5	1	1	2		2	1		3	2	
X																			
Y				1	4	5	3	6	4	2	3	4	8	4	8	3	5	8	2
Z																			
-						1	2	4	6	9	11	16	23	27	29	34	31	14	4
unknown (?)														1			1	1	1
not sequenced			1	1	1	1	1	2	3	3	6	7	8	9	9	10	11	11	11
sum of seq <sup>2</sup>	57	57	56	56	56	56	56	55	54	54	51	50	49	48	48	47	46	46	46
oomcaa <sup>1</sup>	56	54	25	12	10	8	10	11	7	9	11	16	23	27	29	34	31	14	41
mcaa <sup>4</sup>	A	R	G	R	G	G	G	G	V	-	-	-	-	-	-	-	-	-	F D
rel. oomcaa <sup>5</sup>	98%	95%	45%	21%	18%	14%	18%	20%	13%	17%	22%	32%	47%	56%	60%	72%	67%	30%	67%
pos occupied <sup>6</sup>	2	4	12	16	16	16	16	16	16	18	18	13	15	13	10	9	8	5	4

Table 6E: Analysis of V heavy chain subgroup 4

Framework IV													
amino acid <sup>1</sup>	102	103	104	105	106	107	108	109	110	111	112	113	sum
A						1			1				332
B													
C													113
D													210
E													176
F													135
G			41		40	1							674
H	1								1				45
I	9					1							282
K				3									278
L	4						19						540
M							9						43
N						1							204
P	3			2								2	281
Q				29									334
R	1			4			1						250
S	1			1							36	33	986
T				1		33	8		34				532
V	12							36		36			488
W		46											267
X													
Y	16												455
Z													1
-													466
unknown (?)													4
not sequenced	10	11	16	17	17	20	20	21	21	21	21	22	426
sum of seq <sup>2</sup>	47	46	41	40	40	37	37	36	36	36	36	35	
oomcaa <sup>3</sup>	16	46	41	29	40	33	19	36	34	36	36	33	
mcaa <sup>4</sup>	Y	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	34%	100%	100%	73%	100%	89%	51%	100%	94%	100%	100%	94%	
pos occupied <sup>6</sup>	8	1	1	6	1	5	4	1	3	1	1	2	



Table 6F: Analysis of V heavy chain subgroup 5

amino acid <sup>1</sup>	Framework I																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A					1			1	89		1			1						
B																				
C							1													
D										2										
E	88	1			2				4	93						92				
F																	1			
G	1							92							94					
H																				
I																				96
K											94	94							77	
L		1		91		2												95		
M											3								1	
N																				
P				1					1					94						
Q	3		92		1	90										3			1	
R						1						1	1		1				17	
S							92										94			
T																				
V		90			89				1		91									
W																				
X																				
Y																				
Z																				
-																				
unknown (?)																				
not sequenced	5	5	5	5	4	4	4	4	4	2	2	2	2	2	2	2	2	2	1	1
sum of seq <sup>2</sup>	92	92	92	92	93	93	93	93	95	95	95	95	95	95	95	95	95	95	96	96
oomcaa <sup>3</sup>	88	90	92	91	89	90	92	92	89	93	91	94	94	94	94	92	94	95	77	96
mcaa <sup>4</sup>	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	E	S	L	K	I
rel. oomcaa <sup>5</sup>	96%	98%	100%	99%	96%	97%	99%	99%	94%	98%	96%	99%	99%	99%	99%	97%	99%	100%	80%	100%
pos occupied <sup>6</sup>	3	3	1	2	4	3	2	2	4	2	3	2	2	2	2	2	2	1	4	1

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Table 6F: Analysis of V heavy chain subgroup 5

amino acid <sup>1</sup>											CDRI									
	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38
A				3	2					4							8		1	
B																				
C		96						1		1										
D								2		2							1			
E					2					1										
F					3		6		97					2						
G				92		93				1							72			
H										1				4						1
I										4						93				
K			89					1												
L																1			2	
M			1														1		1	
N			1					2		4	14			2						
P					1															1
Q			4																	
R			1			1		2								1				95
S	94			1	90			84		10	61			2	2		15			
T	2							5		75	16					2	1			
V																				
W																1			93	
X																93		97		
Y																				
Z							90							87						
-												97	97							
unknown (?)																				
not sequenced	1	1	1	1	1	1	1													
sum of seq <sup>2</sup>	96	96	96	96	96	96	96	97	97	97	97	97	97	97	97	97	97	97	97	97
oomcaa <sup>1</sup>	94	96	89	92	90	93	90	84	97	75	61	97	97	87	93	93	72	97	93	95
mcaa <sup>4</sup>	S	C	K	G	S	G	Y	S	F	T	S	-	-	Y	W	I	G	W	V	R
rel. oomcaa <sup>5</sup>	98%	100%	93%	96%	94%	97%	94%	87%	100%	77%	63%	100%	100%	90%	96%	96%	74%	100%	96%	98%
pos occupied <sup>6</sup>	2	1	5	3	4	3	2	7	1	5	8	1	1	5	4	4	5	1	4	3

Table 6F: Analysis of V heavy chain subgroup 5

	Framework II																							
amino acid <sup>1</sup>	39	40	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55				
A			1			1									1			2	1					
B																								
C														1				1						
D														14				8	93					
E					3			97											2					
F												1		2										
G				97		96					95							69	1					
H														3	1									
I										1		75	92											
K		1			94																			
L						94				2		2	1											
M		92								89			1											
N																								
P			96				2							1	93					1				
Q	97						1																	
R		1									1	14						1						
S												1			1			16		96				
T		1										3	1		1									
V		2								5	1	1	2											
W									94															
X																								
Y									3					76										
Z																								
-															97 97									
unknown (?)																								
not sequenced																								
sum of seq <sup>2</sup>	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97				
oomcaa <sup>1</sup>	97	92	96	97	94	96	94	97	94	89	95	75	92	76	93	97	97	69	93	96				
mcaa <sup>1</sup>	Q	M	P	G	K	G	L	E	W	M	G	I	I	Y	P	-	-	G	D	S				
rel. oomcaa <sup>3</sup>	100%	95%	99%	100%	97%	99%	97%	100%	97%	92%	98%	77%	95%	78%	96%	100%	100%	71%	96%	99%				
pos occupied <sup>4</sup>	1	5	2	1	2	2	3	1	2	4	3	7	5	6	5	1	1	6	4	2				

Table 6F: Analysis of V heavy chain subgroup 5

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A		6					1										88								
B																									
C					1					1															
D	77									2							97								
E	3									2									2						
F				2				91				1		3											
G	1									94															
H											15														
I		4	1					1				3		88						91					
K			2															93							
L						1		4							2										
M														3						1					
N	2		14	2																					
P						95	1		1										1						
Q									91		81							1							
R			78						3	1				1				1							
S	2	2			95	1	95	1					1		95				96	1					
T		85	2		1								96							4					
V				1								93		2		9									
W																									
X																									
Y	12			92																					
Z																									
-																									
unknown (?)																									
not sequenced																									
sum of seq <sup>2</sup>	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97					
oomcaa <sup>3</sup>	77	85	78	92	95	95	95	91	91	94	81	93	96	88	95	88	97	93	96	91					
mcaa <sup>4</sup>	D	T	R	Y	S	P	S	F	Q	G	Q	V	T	I	S	A	D	K	S	I					
rel. oomcaa <sup>5</sup>	79%	88%	80%	95%	98%	98%	98%	94%	94%	97%	84%	96%	99%	91%	98%	91%	100%	96%	99%	94%					
pos occupied <sup>6</sup>	6	4	5	4	3	3	3	4	4	3	3	3	2	5	2	2	1	4	2	4					

Table 6F: Analysis of V heavy chain subgroup 5

Framework III																				
amino acid <sup>1</sup>	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92
A		1	91								1	96				93				
B																				
C							1													95
D				1										96						
E						1					1									
F				1														2	6	
G								3	1							4				
H						3														
I															2		9			
K											91						1			
L					96					97							2			
M																	84			
N	7							2	2						2					
P			1																	
Q						93														
R	1						1	1	3		3									
S	87	2	1	1				90	91				96		5					
T	2	94	2					1			1	1	1		88		1			
V			2		1									1						
W							95													
X																				
Y				94														94	89	
Z																				
-																				
unknown (?)																				
not sequenced																		1	2	2
sum of seq <sup>2</sup>	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	97	96	95	95
oomcaa <sup>3</sup>	87	94	91	94	96	93	95	90	91	97	91	96	96	96	88	93	84	94	89	95
mcaa <sup>4</sup>	S	T	A	Y	L	Q	W	S	S	L	K	A	S	D	T	A	M	Y	Y	C
rel. oomcaa <sup>5</sup>	90%	97%	94%	97%	99%	96%	98%	93%	94%	100%	94%	99%	99%	99%	91%	96%	87%	98%	94%	100%
pos occupied <sup>6</sup>	4	3	5	4	2	3	3	5	4	1	5	2	2	2	4	2	5	2	2	1

Table 6F: Analysis of V heavy chain subgroup 5

amino acid <sup>1</sup>	CDR III																		
	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K
A	92		1	1	2		3	4	3	2		1			1			4	2
B																			
C						1	1	1			2		1						
D			3	3	3	3	1	2	1	1	2		2	1	1	2			37
E			1	1	1	2			1	1				1			1		
F					1		3			3	2		1						26
G			1	9	11	12	12	5	2	4	3	10	2	1				5	
H			10	1		2			1	1		1							
I				3		2	2	1	1	4	1	1		1	1				
K		1	1	1		1	3	1								2			
L			11	2	3	1	1	2	5		1		1		1				
M					2	1	1		1	1	1	1						10	
N				1		2		1	1	2			1					2	
P			5	1	4	3	1	2				1	1	1	1				
Q		1	3	2		1	1	4	2	1	2								3
R	92	7	9	2	2		2	1		2									
S		1	1	3	2	6	4	4	5	3	5	3	2	2			1	1	
T	1		1	3	2	1	2	6	3	3	6	1		1					
V	2		2	4	4		1		1	2			1						
W			1		2	1					1		2		1		1	1	
X																			
Y				1	6	3	6	9	8	7	2	1	2	6	8	9	9	10	1
Z																			
-						1	1	2	8	10	16	23	30	30	31	32	30	22	7
unknown (?)													1			1	1	1	
not sequenced	2	2	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	53	52
sum of seq <sup>2</sup>	95	95	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	44	45
oomcaa <sup>3</sup>	92	92	11	9	11	12	12	9	8	10	16	23	30	30	31	32	30	22	26
mcaa <sup>4</sup>	A	R	L	G	G	G	G	Y	Y	-	-	-	-	-	-	-	-	F	D
rel. oomcaa <sup>5</sup>	97%	97%	24%	20%	24%	27%	27%	20%	18%	22%	36%	51%	67%	67%	69%	71%	67%	49%	59%
pos occupied <sup>6</sup>	3	4	13	16	14	18	16	15	16	15	14	11	11	9	8	4	6	6	4

Table 6F: Analysis of V heavy chain subgroup 5

Framework IV													
amino acid <sup>1</sup>	102	103	104	105	106	107	108	109	110	111	112	113	sum
A												1	611
B													
C													205
D	1												458
E				1									404
F	2												256
G			41		41								1065
H													44
I	9								2				588
K				3									650
L	2						25	1					549
M							8						303
N													64
P	2					1					1		414
Q				34									612
R				3									351
S	2										40	39	1545
T	1					40	8		39				604
V	11							40		41			594
W		43											432
X													
Y	13												738
Z													
-	2												635
unknown (?)													4
not sequenced	52	54	56	56	56	56	56	56	56	56	56	57	1678
sum of seq <sup>2</sup>	45	43	41	41	41	41	41	41	41	41	41	40	
oomcaa <sup>3</sup>	13	43	41	34	41	40	25	40	39	41	40	39	
mcaa <sup>4</sup>	Y	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	29%	100%	100%	83%	100%	98%	61%	98%	95%	100%	98%	98%	
pos occupied <sup>6</sup>	10	1	1	4	1	2	3	2	2	1	2	2	

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Table 6G: Analysis of V heavy chain subgroup 6

amino acid <sup>1</sup>	Framework I																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A												1								
B																				
C																				
D																				
E																				
F																				
G								52		67										
H																				
I																				
K													68							
L				52							68	1						67	1	68
M																				
N																				
P									68					67					1	
Q	52		52		51	52										68				
R					1					1										
S							52							1	68				66	
T																	68			
V		52										66							1	
W																				
X																				
Y																				
Z																				
-																				
unknown (?)																				
not sequenced	22	22	22	22	22	22	22	22	6	6	6	6	6	6	6	6	6	6	6	6
sum of seq <sup>2</sup>	52	52	52	52	52	52	52	52	68	68	68	68	68	68	68	68	68	68	68	68
oomcaa <sup>3</sup>	52	52	52	52	51	52	52	52	68	67	68	66	68	67	68	68	68	67	66	68
mcaa <sup>4</sup>	Q	V	Q	L	Q	Q	S	G	P	G	L	V	K	P	S	Q	T	L	S	L
rel. oomcaa <sup>5</sup>	100%	100%	100%	100%	98%	100%	100%	100%	100%	99%	100%	97%	100%	99%	100%	100%	100%	99%	97%	100%
pos occupied <sup>6</sup>	1	1	1	1	2	1	1	1	1	2	1	3	1	2	1	1	1	2	3	1



Table 6G: Analysis of V heavy chain subgroup 6

amino acid <sup>1</sup>	CDRI																													
	21	22	23	24	25	26	27	28	29	30	31	A	B	32	33	34	35	36	37	38										
A	1	67												66	67															
B																														
C		68																												
D							68				1							1												
E																														
F										2				1	1					1										
G			1			69							3	1	2															
H																		1												
I				64								2						1		70										
K												3																		
L																														
M																														
N							1				2	66						70												
P																														
Q																														
R											2	1								74										
S	1			1	69			69		68	66		67			3		1												
T	67										2	1	4			1														
V			1	4					70						6					2										
W		1															74		74											
X																														
Y												1								1										
Z																														
-																														
unknown (?)											1																			
not sequenced	5	5	5	5	5	5	5	5	5	4	4																			
sum of seq <sup>2</sup>	69	69	69	69	69	69	69	69	70	70	74	74	74	74	74	74	74	74	74	74										
oomcaa <sup>3</sup>	67	68	67	64	69	69	68	69	70	68	66	66	67	66	67	74	70	74	70	74										
mcaa <sup>4</sup>	T	C	A	I	S	G	D	S	V	S	S	N	S	A	A	W	N	W	I	R										
rel. oomcaa <sup>5</sup>	97%	99%	97%	93%	100%	100%	99%	100%	100%	97%	89%	89%	91%	89%	91%	100%	95%	100%	95%	100%										
pos occupied <sup>6</sup>	3	2	3	3	1	1	2	1	1	2	5	6	3	4	5	1	5	1	4	1										

Table 6G: Analysis of V heavy chain subgroup 6

	Framework II																							
amino acid <sup>1</sup>	39	40	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55				
A				1									1					1						
B																								
C																								
D																								
E								74																
F														2	1			1						
G						74					74	1							1					
H															1									
I																								
K	1				1											1			66					
L	1					74			74															
M																								
N																			1					
P			73																					
Q	72																							
R					73						73					72			1	1				
S		74	1	73												1		72						
T												73							5					
V																								
W								74												73				
X																								
Y														72	72									
Z																								
-																	74							
unknown (?)																								
not sequenced																								
sum of seq <sup>2</sup>	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74				
oomcaa <sup>3</sup>	72	74	73	73	73	74	74	74	74	74	74	73	73	72	72	72	74	72	66	73				
mcaa <sup>4</sup>	Q	S	P	S	R	G	L	E	W	L	G	R	T	Y	Y	R	-	S	K	W				
rel. oomcaa <sup>5</sup>	97%	100%	99%	99%	99%	100%	100%	100%	100%	100%	100%	99%	99%	97%	97%	97%	100%	97%	89%	99%				
pos occupied <sup>6</sup>	3	1	2	2	2	1	1	1	1	1	1	2	2	2	3	3	1	3	5	2				

Table 6G: Analysis of V heavy chain subgroup 6

	CDR II																								
amino acid <sup>1</sup>	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75					
A					73	1							2			6		1							
B																									
C				1																					
D			68			1									2		73								
E	1		3			7			1											2					
F	7																								
G			1				1			8															
H	1																1								
I						1						65	2	71				1							
K		1							67						1					70					
L	1					5		2				4						1							
M												1													
N	2	65	1						1						69										
P						1	1									66									
Q									2		1														
R		1							3		73														
S	2	2	1	1			73			66			1		2	1			73						
T		4											69	1				71	1	2					
V						58		72				4		2		1									
W																									
X																									
Y	60	1		72																					
Z																									
-																									
unknown (?)																									
not sequenced																									
sum of seq <sup>2</sup>	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74	74					
oomcaa <sup>1</sup>	60	65	68	72	73	58	73	72	67	66	73	65	69	71	69	66	73	71	73	70					
mcaa <sup>1</sup>	Y	N	D	Y	A	V	S	V	K	S	R	I	T	I	N	P	D	T	S	K					
rel. oomcaa <sup>3</sup>	81%	88%	92%	97%	99%	78%	99%	97%	91%	89%	99%	88%	93%	96%	93%	89%	99%	96%	99%	95%					
pos occupied <sup>4</sup>	7	6	5	3	2	7	2	2	5	2	2	4	4	3	4	4	2	4	2	3					

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Table 6G: Analysis of V heavy chain subgroup 6

Framework III																				
amino acid <sup>1</sup>	76	77	78	79	80	81	82	A	B	C	83	84	85	86	87	88	89	90	91	92
A													1		74					
B																				
C																				73
D								3						73						
E													73							
F			71						1										3	
G														1						
H						2		1												
I			1														2			
K								4												
L		1			74		72													
M							1			1							2			
N	74							63											1	
P												70								
Q		72				71														
R		1				1		1												1
S				74				1	73		1	3								
T								1			73				74			1		
V			2				1			73							70			
W																				
X																				
Y																		73	70	
Z																				
-																				
unknown (?)																				
not sequenced											1									
sum of seq <sup>2</sup>	74	74	74	74	74	74	74	74	74	74	74	73	74	74	74	74	74	74	74	74
oomcaa <sup>3</sup>	74	72	71	74	74	71	72	63	73	73	73	70	73	73	74	74	70	73	70	73
mcaa <sup>4</sup>	N	Q	F	S	L	Q	L	N	S	V	T	P	E	D	T	A	V	Y	Y	C
rel. oomcaa <sup>5</sup>	100%	97%	96%	100%	100%	96%	97%	85%	99%	99%	99%	96%	99%	99%	100%	100%	95%	99%	95%	99%
pos occupied <sup>6</sup>	1	3	3	1	1	3	3	7	2	2	2	2	2	2	1	1	3	2	3	2

Table 6G: Analysis of V heavy chain subgroup 6

amino acid <sup>1</sup>	CDR III																			
	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K	101
A	69		11	1	3	12	4	3	2	5		8						10	1	
B																				
C					1		1			1		1	1							
D			19	4	3	7	4	3	1	6	1	1	1							62
E			10	4	2	1	2	2	1	2							1			
F	1		1	1	1		1	2	3		2			1					38	4
G	1		16	4	15	15	11	8	6	2	5	1	8	6	1			17		
H				1		1			1	1	1	1				1	1	1		
I				1	2		2		5	1										
K		1	1	1	1	1	1	1				1								
L			1	8	4	2	3	2	1					1	5				8	
M				1				1			5								11	
N			1	3	1	2	1	1	1	3		2		1		1	3			
P				10	4		5	3		5	1		1							
Q			1	1	1	1					1									1
R		69	1	7	8	1	8	8	3		1	1	5							1
S		3	5	5	5	7	6	7	3	4	2					1	1			
T			1	1	4	3	4	4	6	3	1			1						
V	3	1	4	5	1	9			4		9	5	1	1					2	
W			1	6	8		3	2	4								4	4		
X																				
Y				6	4	2	2	2	6	6	2	4	2	1	8	8	12	12		
Z																				
-				2	3	7	14	23	25	33	41	47	53	54	57	56	50	28	12	4
unknown (?)														6	1	5				
not sequenced				1	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
sum of seq <sup>2</sup>	74	74	73	72	71	71	72	72	72	72	72	72	72	72	72	72	72	72	72	72
oomcaa <sup>3</sup>	69	69	19	10	15	15	14	23	25	33	41	47	53	54	57	56	50	28	38	62
mcaa <sup>4</sup>	A	R	D	P	G	G	-	-	-	-	-	-	-	-	-	-	-	-	F	D
rel. oomcaa <sup>5</sup>	93%	93%	26%	14%	21%	21%	19%	32%	35%	46%	57%	65%	74%	75%	79%	78%	69%	39%	53%	86%
pos occupied <sup>6</sup>	4	4	14	20	19	15	17	16	16	13	13	11	8	8	4	5	7	6	6	5

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Table 6G: Analysis of V heavy chain subgroup 6

amino acid <sup>1</sup>	Framework IV												sum
	102	103	104	105	106	107	108	109	110	111	112	113	
A							2						494
B													
C													147
D								1					403
E													186
F	2										2		150
G			49		50								571
H	2												18
I	9					3		1					304
K				1			1						293
L	5						26						632
M							8						31
N													436
P	4			6								1	387
Q				40									539
R				2									495
S	4		1			1					43	46	1271
T						45	4		45				640
V	21						2	46		48			647
W		65					5						398
X													
Y	19												518
Z													
-	2												585
unknown (?)													13
not sequenced <sup>2</sup>	5	8	23	24	23	24	25	25	28	25	28	26	580
sum of seq <sup>3</sup>	68	65	50	49	50	49	48	48	45	48	45	47	
oomcaa <sup>3</sup>	21	65	49	40	50	45	26	46	45	48	43	46	
mcaa <sup>4</sup>	V	W	G	Q	G	T	L	V	T	V	S	S	
rel. oomcaa <sup>5</sup>	31%	100%	98%	82%	100%	92%	54%	96%	100%	100%	96%	98%	
pos occupied <sup>6</sup>	9	1	2	4	1	3	7	3	1	1	2	2	

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## Appendix to Tables 1A-C

A. *References of rearranged sequences**References of rearranged human kappa sequences used for alignment*

- 1 Alescio-Zonta, L. & Baglioni, C. (1970) Eur.J.Biochem., 15, 450-463.
- 2 Andrews, D.W. & Capra, J.D. (1981) Biochemistry, 20, 5816-5822.
- 3 Andris, J.S., Ehrlich, P.H., Ostberg, L. & Capra, J.D. (1992) J.Immunol., 149, 4053-4059.
- 4 Atkinson, P.M., Lampman, G.W., Furie, B.C., Naparstek, Y., Schwartz, R.S., Stollar, B.D. & Furie, B. (1985) J.Clin.Invest., 75, 1138-1143.
- 5 Aucouturier, P., Bauwens, M., Khamlichi, A.A., Denoroy, L., Spinelli, S., Touchard, G., Preud'homme, J.-L. & Cogne, M. (1993) J.Immunol., 150, 3561-3568.
- 6 Avila, M.A., Vazques, J., Danielsson, L., Fernandez De Cossio, M.E. & Borrebaeck, C.A.K. (1993) Gene, 127, 273-274.
- 7 Barbas III, C.F., Crowe, Jr., J.E., Cababa, D., Jones, T.M., Zebedee, S.L., Murphy, B.R., Chanock, R.M. & Burton, D.R. (1992) Proc.Natl.Acad.Sci.Usa, 89, 10164-10168.
- 8 Barbas, C.F., III, et al. (1993) J-Mol-Biol., 230, 812-23.
- 9 Bentley, D.L. & Rabbitts, T.H. (1980) Nature, 288, 730-733.
- 10 Bentley, D.L. & Rabbitts, T.H. (1983) Cell, 32, 181-189.
- 11 Bentley, D.L. (1984) Nature, 307, 77-80.
- 12 Bhat, N.M., Bieber, M.M., Chapman, C.J., Stevenson, F.K. & Teng, N.N.H. (1993) J.Immunol., 151, 5011-5021.
- 13 Blaison, G., Kuntz, J.-L. & Pasquali, J.-L. (1991) Eur.J.Immunol., 21, 1221-1227.
- 14 Braun, H., Leibold, W., Barnikol, H.U. & Hilschmann, N. (1971) Z.Physiol.Chem., 352, 647-651; (1972) Z.Physiol.Chem., 353, 1284-1306.
- 15 Capra, J.D. & Kehoe, J.M. (1975) Adv.Immunology, 20, 1-40. ; Andrews, D.W. & Capra, J.D. (1981) Proc.Natl.Acad.Sci.Usa, 78, 3799-3803.
- 16 Capra, J.D. & Kehoe, J.M. (1975) Adv.Immunology, 20, 1-40. ; Ledford, D.K., Goni, F., Pizzolato, M., Franklin, E.C., Solomon, A. & Frangione, B. (1983) J.Immunol., 131, 1322-1325.
- 17 Chastagner, P., Theze, J. & Zouali, M. (1991) Gene, 101, 305-306.

- 18 Chen, P.P., Robbins, D.L., Jirik, F.R., Kipps, T.J. & Carson, D.A. (1987) *J.Exp.Med.*, 166, 1900-1905.
- 19 Chen, P.P., Robbins, D.L., Jirik, F.R., Kipps, T.J. & Carson, D.A. (1987) *J.Exp.Med.*, 166, 1900-1905; Liu, M.-F., Robbins, D.L., Crowley, J.J., Sinha, S., Kozin, F., Kipps, T.J., Carson, D.A. & Chen, P.P. (1989) *J.Immunol.*, 142, 688-694.
- 20 Chersi, A. & Natali, P.G. (1978) *Immunochemistry*, 15, 585-589.
- 21 Co, M.S., Deschamps, M., Whitley, R.J. & Queen, C. (1991) *Proc.Natl.Acad.Sci.Usa*, 88, 2869-2873.
- 22 Cuisinier, A.-M., Fumoux, F., Fougereau, M. & Tonnelle, C. (1992) *Mol.Immunol.*, 29, 1363-1373.
- 23 Davidson, A., Manheimer-Lory, A., Aranow, C., Peterson, R., Hannigan, N. & Diamond, B. (1990) *J.Clin.Invest.*, 85, 1401-1409.
- 24 Denomme, G.A., Mahmoudi, M., Edwards, J.Y., Massicotte, H., Cairns, E. & Bell, D.A. (1993) *Hum.Antibod.Hybridomas*, 4, 98-103.
- 25 Dersimonian, H., Mcadam, K.P.W.J., Mackworth-Young, C. & Stollar, B.D. (1989) *J.Immunol.*, 142, 4027-4033.
- 26 Dreyer, W.J., Gray, W.R. & Hood, L. (1967) *Cold Spring Harbor Symp. Quantitative Biol.*, 32, 353-367.
- 27 Ebeling, S.B., Schutte, M.E.M. & Logtenberg, T. (1993) *Eur.J.Immunol.*, 23, 1405-1408.
- 28 Eulitz, M. & Kley, H.-P. (1977) *Immunochem.*, 14, 289-297.
- 29 Eulitz, M. & Linke, R.P. (1982) *Z.Physiol.Chem.*, 363, 1347-1358.
- 30 Eulitz, M., Breuer, M., Eblen, A., Weiss, D.T. & Solomon, A. (1990) In *Amyloid And Amyloidosis*, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 31 Eulitz, M., Gotze, D. & Hilschmann, N. (1972) *Z.Physiol.Chem.*, 353, 487-491; Eulitz, M. & Hilschmann, N. (1974) *Z.Physiol.Chem.*, 355, 842-866.
- 32 Eulitz, M., Kley, H.P. & Zeitler, H.J. (1979) *Z.Physiol.Chem.*, 360, 725-734.
- 33 Ezaki, I., Kanda, H., Sakai, K., Fukui, N., Shingu, M., Nobunaga, M. & Watanabe, T. (1991) *Arthritis And Rheumatism*, 34, 343-350.
- 34 Felgenhauer, M., Kohl, J. & Ruker, F. (1990) *Nucl.Acids Res.*, 18, 4927.
- 35 Ferri, G., Stoppini, M., Iadarola, P., Bellotti, V. & Merlini, G. (1989) *Biochim.Biophys.Acta*, 995, 103-108.



- 36 Gillies, S.D., Dorai, H., Wesolowski, J., Majeau, G., Young, D., Boyd, J., Gardner, J. & James, K. (1989) *Bio/Tech.*, 7, 799-804.
- 37 Goni, F. & Frangione, B. (1983) *Proc.Nat.Acad.Sci.Usa*, 80, 4837-4841.
- 38 Goni, F.R., Chen, P.P., McGinnis, D., Arjonilla, M.L., Fernandez, J., Carson, D., Solomon, A., Mendez, E. & Frangione, B. (1989) *J.Immunol.*, 142, 3158-3163.
- 39 Gorman, S.D., Clark, M.R., Routledge, E.G., Cobbold, S.P. & Waldmann, H. (1991) *Proc.Natl.Acad.Sci.Usa*, 88, 4181-4185.
- 40 Gottlieb, P.D., Cunningham, B.A., Rutishauser, U. & Edelman, G.M. (1970) *Biochemistry*, 9, 3155-3161.
- 41 Griffiths, A.D., Malmqvist, M., Marks, J.D., Bye, J.M., Embleton, M.J., Mccafferty, J., Baier, M., Holliger, K.P., Gorick, B.D., Hughes-Jones, N.C., Hoogenboom, H.R. & Winter, G. (1993) *Embo J.*, 12, 725-734.
- 42 Hieter, P.A., Max, E.E., Seidman, J.G., Maizel, J.V., Jr. & Leder, P. (1980) *Cell*, 22, 197-207; Klobeck, H.G, Meindl, A., Combriato, G., Solomon, A. & Zachau, H.G. (1985) *Nucl.Acids Res.*, 13, 6499-6513; Weir, L. & Leder, P. (1986)
- 43 Hilschmann, N. & Craig, L.C. (1965) *Proc.Nat.Acad.Sci.Usa*, 53, 1403-1409; Hilschmann, N. (1967) *Z.Physiol.Chem.*, 348, 1077-1080.
- 44 Hilschmann, N. & Craig, L.C. (1965) *Proc.Nat.Acad.Sci.Usa*, 53, 1403-1409; Hilschmann, N. (1967) *Z.Physiol.Chem.*, 348, 1718-1722; Hilschmann, N. (1969) *Naturwissenschaften*, 56, 195-205.
- 45 Hirabayashi, Y., Munakata, Y., Sasaki, T. & Sano, H. (1992) *Nucl.Acids Res.*, 20, 2601.
- 46 Jaenichen, H.-R., Pech, M., Lindenmaier, W., Wildgruber, N. & Zachau, H.G. (1984) *Nuc.Acids Res.*, 12, 5249-5263.
- 47 Jirik, F.R., Sorge, J., Fong, S., Heitzmann, J.G., Curd, J.G., Chen, P.P., Goldfien, R. & Carson, D.A. (1986) *Proc.Nat.Acad.Sci.Usa*, 83, 2195-2199.
- 48 Kaplan, A.P. & Metzger, H. (1969) *Biochemistry*, 8, 3944-3951. ; Klapper, D.G. & Capra, J.D. (1976) *Ann.Immunol.(Inst.Pasteur)*, 127c, 261-271.
- 49 Kennedy, M.A. (1991) *J.Exp.Med.*, 173, 1033-1036.
- 50 Kim, H.S. & Deutsch, H.F. (1988) *Immunol.*, 64, 573-579.
- 51 Kipps, T.J., Tomhave, E., Chen, P.P. & Carson, D.A. (1988) *J.Exp.Med.*, 167, 840-852.
- 52 Kipps, T.J., Tomhave, E., Chen, P.P. & Fox, R.I. (1989) *J.Immunol.*, 142, 4261-4268.
- 53 Klapper, D.G. & Capra, J.D. (1976) *Ann.Immunol.(Inst.Pasteur)*, 127c, 261-271.

195

- 54 Klein, U., Kuppers, R. & Rajewsky, K. (1993) *Eur.J.Immunol.*, 23, 3272-3277.
- 55 Klobeck, H.G, Meindl, A., Combriato, G., Solomon, A. & Zachau, H.G. (1985) *Nucl.Acids Res.*, 13, 6499-6513.
- 56 Klobeck, H.G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.D. & Zachau, H.G. (1985) *Nucl.Acids Res.*, 13, 6515-6529.
- 57 Klobeck, H.G., Combriato, G. & Zachau, H.G. (1984) *Nuc.Acids Res.*, 12, 6995-7006.
- 58 Klobeck, H.G., Solomon, A. & Zachau, H.G. (1984) *Nature*, 309, 73-76.
- 59 Knight, G.B., Agnello, V., Bonagura, V., Barnes, J.L., Panka, D.J. & Zhang, Q.-X. (1993) *J.Exp.Med.*, 178, 1903-1911.
- 60 Kohler, H., Shimizu, A., Paul, C. & Putnam, F.W. (1970) *Science*, 169, 56-59. (Kaplan, A.P. & Metzger, H. (1969) *Biochemistry*, 8, 3944-3951.)
- 61 Kratzin, H., Yang, C.Y., Krusche, J.U. & Hilschmann, N. (1980) *Z.Physiol.Chem.*, 361, 1591-1598.
- 62 Kunicki, T.J., Annis, D.S., Gorski, J. & Nugent, D.J. (1991) *J.Autoimmunity*, 4, 433-446.
- 63 Larrick, J.W., Wallace, E.F., Coloma, M.J., Bruderer, U., Lang, A.B. & Fry, K.E. (1992) *Immunological Reviews*, 130, 69-85.
- 64 Laure, C.J., Watanabe, S. & Hilschmann, N. (1973) *Z.Physiol.Chem.*, 354, 1503-1504.
- 65 Ledford, D.K., Goni, F., Pizzolato, M., Franklin, E.C., Solomon, A. & Frangione, B. (1983) *J.Immunol.*, 131, 1322-1325.
- 66 Ledford, D.K., Goni, F., Pizzolato, M., Franklin, E.C., Solomon, A. & Frangione, B. (1983) *J.Immunol.*, 131, 1322-1325.
- 67 Ledford, D.K., Goni, F., Pizzolato, M., Franklin, E.C., Solomon, A. & Frangione, B. (1983) *J.Immunol.*, 131, 1322-1325. Pons-Estel, B., Goni, F., Solomon, A. & Frangione, B. (1984) *J.Exp.Med.*, 160, 893.
- 68 Levy, S., Mendel, E., Kon, S., Avnur, Z. & Levy, R. (1988) *J.Exp.Med.*, 168, 475-489.
- 69 Liepnieks, J.J., Dwulet, F.E. & Benson, M.D. (1990) *Mol.Immunol.*, 27, 481-485.
- 70 Manheimer-Lory, A., Katz, J.B., Pillinger, M., Ghossein, C., Smith, A. & Diamond, B. (1991) *J.Exp.Med.*, 174, 1639-1652.
- 71 Mantovani, L., Wilder, R.L. & Casali, P. (1993) *J.Immunol.*, 151, 473-488.
- 72 Mariette, X., Tsapis, A. & Brouet, J.-C. (1993) *Eur.J.Immunol.*, 23, 846-851.
- 73 Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., Mccafferty, J., Griffiths, A.D. & Winter, G. (1991) *J.Mol.Biol.*, 222, 581-597.

196

- 74 Marsh, P., Mills, F. & Gould, H. (1985) *Nuc.Acids Res.*, 13, 6531-6544.
- 75 Middaugh, C.R. & Litman, G.W. (1987) *J.Biol.Chem.*, 262, 3671-3673.
- 76 Milstein, C. & Deverson, E.V. (1971) *Biochem.J.*, 123, 945-958.
- 77 Milstein, C. (1969) *Febs Letters*, 2, 301-304.
- 78 Milstein, C. (1969) *Febs Letters*, 2, 301-304.
- 79 Milstein, C.P. & Deverson, E.V. (1974) *Eur.J.Biochem.*, 49, 377-391.
- 80 Moran, M.J., Andris, J.S., Matsumoto, Y.-I., Capra, J.D. & Hersh, E.M. (1993) *Mol.Immunol.*, 30, 1543-1551.
- 81 Nakatani, T., Nomura, N., Horigome, K., Ohtsuka, H. & Noguchi, H. (1989) *Bio/Tech.*, 7, 805-810.
- 82 Newkirk, M., Chen, P.P., Carson, D., Posnett, D. & Capra, J.D. (1986) *Mol.Immunol.*, 23, 239-244.
- 83 Newkirk, M.M., Gram, H., Heinrich, G.F., Ostberg, L., Capra, J.D. & Wasserman, R.L. (1988) *J.Clin.Invest.*, 81, 1511-1518.
- 84 Newkirk, M.M., Mageed, R.A., Jefferis, R., Chen, P.P. & Capra, J.D. (1987) *J.Exp.Med.*, 166, 550-564.
- 85 Olee, B.T., Lu, E.W., Huang, D.-F., Soto-Gil, R.W., Deftos, M., Kozin, F., Carson, D.A. & Chen, P.P. (1992) *J.Exp.Med.*, 175, 831-842.
- 86 Palm, W. & Hilschmann, N. (1973) *Z.Physiol.Chem.*, 354, 1651-1654; (1975) *Z.Physiol.Chem.*, 356, 167-191.
- 87 Pascual, V., Victor, K., Lelsz, D., Spellerberg, M.B., Hamblin, T.J., Thompson, K.M., Randen, I., Natvig, J., Capra, J.D. & Stevenson, F.K. (1991) *J.Immunol.*, 146, 4385-4391.
- 88 Pascual, V., Victor, K., Randen, I., Thompson, K., Steinitz, M., Forre, O., Fu, S.-M., Natvig, J.B. & Capra, J.D. (1992) *Scand.J.Immunol.*, 36, 349-362.
- 89 Pech, M. & Zachau, H.G. (1984) *Nuc.Acids Res.*, 12, 9229-9236.
- 90 Pech, M., Jaenichen, H.-R., Pohlenz, H.-D., Neumaier, P.S., Klobeck, H.-G. & Zachau, H.G. (1984) *J.Mol.Biol.*, 176, 189-204.
- 91 Pons-Estel, B., Goni, F., Solomon, A. & Frangione, B. (1984) *J.Exp.Med.*, 160, 893-904.
- 92 Portolano, S., Mclachlan, S.M. & Rapoport, B. (1993) *J.Immunol.*, 151, 2839-2851.
- 93 Portolano, S., Seto, P., Chazenbalk, G.D., Nagayama, Y., Mclachlan, S.M. & Rapoport, B. (1991) *Biochem.Biophys.Res.Comm.*, 179, 372-377.

197

- 94 Pratt, L.F., Rassenti, L., Larrick, J., Robbins, B., Banks, P.M. & Kipps, T.J. (1989) *J.Immunol.*, 143, 699-705.
- 95 Prelli, F., Tummolo, D., Solomon, A. & Frangione, B. (1986) *J.Immunol.*, 136, 4169-4173.
- 96 Putnam, F.W., Whitley, E.J., Jr., Paul, C. & Davidson, J.N. (1973) *Biochemistry*, 12, 3763-3780.
- 97 Randen, I., Pascual, V., Victor, K., Thompson, K.M., Forre, O., Capra, J.D. & Natvig, J.B. (1993) *Eur.J.Immunol.*, 23, 1220-1225.
- 98 Rassenti, L.Z., Pratt, L.F., Chen, P.P., Carson, D.A. & Kipps, T.J. (1991) *J.Immunol.*, 147, 1060-1066.
- 99 Reidl, L.S., Friedman, D.F., Goldman, J., Hardy, R.R., Jefferies, L.C. & Silberstein, L.E. (1991) *J.Immunol.*, 147, 3623-3631.
- 100 Riechmann, L., Clark, M., Waldmann, H. & Winter, G. (1988) *Nature*, 332, 323-327.
- 101 Riesen, W., Rudikoff, S., Oriol, R. & Potter, M. (1975) *Biochemistry*, 14, 1052-1057; Riesen, W.F., Braun, D.G. & Jaton, J.C. (1976) *Proc.Nat.Acad.Sci.Usa*, 73, 2096-2100; Riesen, W.F. & Jaton, J.C. (1976) *Biochemistry*, 15, 3829.
- 102 Rodilla Sala, E., Kratzin, D.H., Pick, A.I. & Hilschmann, N. (1990) In *Amyloid And Amyloidosis*, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 103 Schiechl, H. & Hilschmann, N. (1971) *Z.Physiol.Chem.*, 352, 111-115; (1972) *Z.Physiol.Chem.*, 353, 345-370.
- 104 Schneider, M. & Hilschmann, N. (1974) *Z.Physiol.Chem.*, 355, 1164-1168.
- 105 Shearman, C.W., Pollock, D., White, G., Hehir, K., Moore, G.P., Kanzy, E.J. & Kurre, R. (1991) *J.Immunol.*, 147, 4366-4373.
- 106 Shinoda, T. (1973) *J.Biochem.*, 73, 433-446.
- 107 Shinoda, T. (1975) *J.Biochem.*, 77, 1277-1296.
- 108 Shinoda, T., Takenawa, T., Hoshi, A. & Isobe, T. (1990) In *Amyloid And Amyloidosis*, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic Publishers, Dordrecht/Boston/London, Pp.157-
- 109 Silberstein, L.E., Litwin, S. & Carmack, C.E. (1989) *J.Exp.Med.*, 169, 1631-1643.
- 110 Sims, M.J., Hassal, D.G., Brett, S., Rowan, W., Lockyer, M.J., Angel, A., Lewis, A.P., Hale, G., Waldmann, H. & Crowe, J.S. (1993) *J.Immunol.*, 151, 2296-2308.

- 111 Spatz, L.A., Wong, K.K., Williams, M., Desai, R., Golier, J., Berman, J.E., Alt, F.W. & Latov, N. (1990) J.Immunol., 144, 2821-2828.
- 112 Stavnezer, J., Kekish, O., Batter, D., Grenier, J., Balazs, I., Henderson, E. & Zegers, B.J.M. (1985) Nucl.Acids Res., 13, 3495-3514.
- 113 Straubinger, B., Thiebe, R., Pech, M. & Zachau, H.G. (1988) Gene, 69, 209-214.
- 114 Suter, L., Barnikol, H.U., Watanabe, S. & Hilschmann, N. (1969) Z.Physiol.Chem., 350, 275-278; (1972) Z.Physiol.Chem., 353, 189-208.
- 115 Tempest, P.R., Bremner, P., Lambert, M., Taylor, G., Furze, J.M., Carr, F.J. & Harris, W.J. (1991) Bio/Tech., 9, 266-271.
- 116 Titani, K., Shinoda, T. & Putnam, F.W. (1969) J.Biol.Chem., 244, 3550-3560.
- 117 Toft, K.G., Olstad, O.K., Sletten, K. & Westermark, P. (1990) In Amyloid And Amyloidosis, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 118 Van Es, J.H., Aanstoot, H., Gmelig-Meyling, F.H.J., Derksen, R.H.W.M. & Logtenberg, T. (1992) J.Immunol., 149, 2234-2240.
- 119 Victor, K.D., Pascual, V., Lefvert, A.K. & Capra, J.D. (1992) Mol.Immunol., 29, 1501-1506.
- 120 Victor, K.D., Pascual, V., Williams, C.L., Lennon, V.A. & Capra, J.D. (1992) Eur.J.Immunol., 22, 2231-2236.
- 121 Victor, K.D., Randen, I., Thompson, K., Forre, O., Natvig, J.B., Fu, S.M. & Capra, J.D. (1991) J.Clin.Invest., 87, 1603-1613.
- 122 Wagner, S.D. & Luzzatto, L. (1993) Eur.J.Immunol., 23, 391-397.
- 123 Watanabe, S. & Hilschmann, N. (1970) Z.Physiol.Chem., 351, 1291-1295.
- 124 Weisbart, R.H., Wong, A.L., Noritake, D., Kacena, A., Chan, G., Ruland, C., Chin, E., Chen, I.S.Y. & Rosenblatt, J.D. (1991) J.Immunol., 147, 2795-2801.
- 125 Weng, N.-P., Yu-Lee, L.-Y., Sanz, I., Patten, B.M. & Marcus, D.M. (1992) J.Immunol., 149, 2518-2529.
- 126 Winkler, T.H., Fehr, H. & Kalden, J.R. (1992) Eur.J.Immunol., 22, 1719-1728.

References of rearranged human lambda sequences used for alignment

- 1 Alexandre, D., Chuchana, P., Brockly, F., Blancher, A., Lefranc, G. & Lefranc, M.-P. (1989) Nuc.Acids Res., 17, 3975.

199

- 2 Anderson, M.L.M., Brown, L., Mckenzie, E., Kellow, J.E. & Young, B.D. (1985) *Nuc.Acids Res.*, 13, 2931-2941.
- 3 Andris, J.S., Brodeur, B.R. & Capra, J.D. (1993) *Mol.Immunol.*, 30, 1601-1616.
- 4 Andris, J.S., Ehrlich, P.H., Ostberg, L. & Capra, J.D. (1992) *J.Immunol.*, 149, 4053-4059.
- 5 Baczko, K., Braun, D.G., Hess, M. & Hilschmann, N. (1970) *Z.Physiol.Chem.*, 351, 763-767; Baczko, K., Braun, D.G. & Hilschmann, N. (1974) *Z.Physiol.Chem.*, 355, 131-154.
- 6 Berinstein, N., Levy, S. & Levy, R. (1989) *Science*, 244, 337-339.
- 7 Bhat, N.M., Bieber, M.M., Chapman, C.J., Stevenson, F.K. & Teng, N.N.H. (1993) *J.Immunol.*, 151, 5011-5021.
- 8 Cairns, E., Kwong, P.C., Misener, V., Ip, P., Bell, D.A. & Siminovitch, K.A. (1989) *J.Immunol.*, 143, 685-691.
- 9 Carroll, W.L., Yu, M., Link, M.P. & Korsmeyer, S.J. (1989) *J.Immunol.*, 143, 692-698.
- 10 Chen, B.L. & Poljak, R.J. (1974) *Biochemistry*, 13, 1295-1302.
- 11 Chen, B.L., Chiu, Y.Y.H., Humphrey, R.L. & Poljak, R.J. (1978) *Biochim.Biophys.Acta*, 537, 9-21.
- 12 Combriato, G. & Klobeck, H.G. (1991) *Eur.J.Immunol.*, 21, 1513-1522.
- 13 Cuisinier, A.-M., Fumoux, F., Fougereau, M. & Tonnelle, C. (1992) *Mol.Immunol.*, 29, 1363-1373.
- 14 Dwulet, F.E., Strako, K. & Benson, M.D. (1985) *Scand.J.Immunol.*, 22, 653-660.
- 15 Elahna, P., Livneh, A., Manheimer-Lory, A.J. & Diamond, B. (1991) *J.Immunol.*, 147, 2771-2776.
- 16 Engelhard, M., Hess, M. & Hilschmann, N. (1974) *Z.Physiol.Chem.*, 355, 85-88; Engelhard, M. & Hilschmann, N. (1975) *Z.Physiol.Chem.*, 356, 1413-1444.
- 17 Eulitz, M. (1974) *Eur.J.Biochem.*, 50, 49-69.
- 18 Eulitz, M., Breuer, M. & Linke, R.P. (1987) *Biol.Che.Hoppe-Seyler*, 368, 863-870.
- 19 Eulitz, M., Murphy, C., Weiss, D.T. & Solomon, A. (1991) *J.Immunol.*, 146, 3091-3096.
- 20 Fett, J.W. & Deutsch, H.F. (1974) *Biochemistry*, 13, 4102-4114.
- 21 Fett, J.W. & Deutsch, H.F. (1976) *Immunochem.*, 13, 149-155.; Jabusch, J.R. & Deutsch, H.F. (1982) *Mol.Immunol.*, 19, 901-906.
- 22 Furey, W. Jr., Wang, B.C., Yoo, C.S. & Sax, M. (1983) *J.Mol.Biol.*, 167, 661-692.
- 23 Fykse, E.-M., Sletten, K., Husby, G. & Cornwell, G.G., Iii (1988) *Biochem.J.*, 256, 973-980.

- 24 Garver, F.A. & Hilschmann, N. (1971) *Febs Letters*, 16, 128-132; (1972) *Eur.J.Biochem.*, 26, 10-32.
- 25 Gawinowicz, M.A., Merlini, G., Birken, S., Osserman, E.F. & Kabat, E.A. (1991) *J.Immunol.*, 147, 915-920.
- 26 Ghiso, J., Solomon, A. & Frangione, B. (1986) *J.Immunol.*, 136, 716-719.
- 27 Griffiths, A.D., Malmqvist, M., Marks, J.D., Bye, J.M., Embleton, M.J., McCafferty, J., Baier, M., Holliger, K.P., Gorick, B.D., Hughes-Jones, N.C., Hoogenboom, H.R. & Winter, G. (1993) *Embo J.*, 12, 725-734.
- 28 Gullasken, N., Idso, H., Nilsen, R., Sletten, K., Husby, G. & Cornwell, G.G. (1990) In *Amyloid And Amyloidosis*, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 29 Harindranath, N., Goldfarb, I.S., Ikematsu, H., Burastero, S.E., Wilder, R.L., Notkins, A.L. & Casali, P. (1991) *Int.Immunol.*, 3, 865-875.
- 30 Holm, E., Sletten, K. & Husby, G. (1986) *Biochem.J.*, 239, 545-551.
- 31 Hughes-Jones, N.C., Bye, J.M., Beale, D. & Coadwell, J. (1990) *Biochem.J.*, 268, 135-140.
- 32 Kametani, F., Yoshimura, K., Tonoike, H., Hoshi, A., Shinoda, T. & Isobe, T. (1985) *Biochem.Biophys.Res.Comm.*, 126, 848-852.
- 33 Kiefer, C.R., Mcguire, B.S., Jr., Osserman, E.F. & Garver, F.A. (1983) *J.Immunol.*, 131, 1871-1875.
- 34 Kiefer, C.R., Patton, H.M., Jr., Mcquire, B.S., Jr. & Garver, F.A. (1980) *J.Immunol.*, 124, 301-306.
- 35 Kishimoto, T., Okajima, H., Okumoto, T. & Taniguchi, M. (1989) *Nucl.Acids Res.*, 17, 4385.
- 36 Klafki, H.-W., Kratzin, H.D., Pick, A.I., Eckart, K. & Hilschmann, N. (1990) In *Amyloid And Amyloidosis*, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 37 Kohler, H., Rudofsky, S. & Kluskens, L. (1975) *J.Immunology*, 114, 415-421.
- 38 Kojima, M., Odani, S. & Ikenaka, T. (1980) *Mol.Immunol.*, 17, 1407-1414.
- 39 Komori, S., Yamasaki, N., Shigeta, M., Isojima, S. & Watanabe, T. (1988) *Clin.Exp.Immunol.*, 71, 508-516.
- 40 Kratzin, H.D., Palm, W., Stangel, M., Schmidt, W.E., Friedrich, J. & Hilschmann, N. (1989) *Biol.Chem.Hoppe-Seyler*, 370, 263-272.

- 41 Kratzin, H.D., Pick, A.I., Stangel, M. & Hilschmann, N. (1990) In Amyloid And Amyloidosis, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic Publishers, Dordrecht/Boston/London, Pp.181-
- 42 Langer, B., Steinmetz-Kayne, M. & Hilschmann, N. (1968) Z.Physiol.Chem., 349, 945-951.
- 43 Larrick, J.W., Danielsson, L., Brenner, C.A., Wallace, E.F., Abrahamson, M., Fry, K.E. & Borrebaeck, C.A.K. (1989) Bio/Tech., 7, 934-938.
- 44 Levy, S., Mendel, E., Kon, S., Avnur, Z. & Levy, R. (1988) J.Exp.Med., 168, 475-489.
- 45 Lewis, A.P., Lemon, S.M., Barber, K.A., Murphy, P., Parry, N.R., Peakman, T.C., Sims, M.J., Worden, J. & Crowe, J.S. (1993) J.Immunol., 151, 2829-2838.
- 46 Liu, V.Y.S., Low, T.L.K., Infante, A. & Putnam, F.W. (1976) Science, 193, 1017-1020; Infante, A. & Putnam, F.W. (1979) J.Biol.Chem., 254, 9006-9016.
- 47 Lopez De Castro, J.A., Chiu, Y.Y.H. & Poljak, R.J. (1978) Biochemistry, 17, 1718-1723.
- 48 Mantovani, L., Wilder, R.L. & Casali, P. (1993) J.Immunol., 151, 473-488.
- 49 Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., Mccafferty, J., Griffiths, A.D. & Winter, G. (1991) J.Mol.Biol., 222, 581-597.
- 50 Mihaesco, E., Roy, J.-P., Congy, N., Peran-Rivat, L. & Mihaesco, C. (1985) Eur.J.Biochem., 150, 349-357.
- 51 Milstein, C., Clegg, J.B. & Jarvis, J.M. (1968) Biochem.J., 110, 631-652.
- 52 Moran, M.J., Andris, J.S., Matsumoto, Y.-I., Capra, J.D. & Hersh, E.M. (1993) Mol.Immunol., 30, 1543-1551.
- 53 Nabeshima, Y. & Ikenaka, T. (1979) Mol.Immunol., 16, 439-444.
- 54 Olee, B.T., Lu, E.W., Huang, D.-F., Soto-Gil, R.W., Deftos, M., Kozin, F., Carson, D.A. & Chen, P.P. (1992) J.Exp.Med., 175, 831-842.
- 55 Pascual, V., Victor, K., Randen, I., Thompson, K., Steinitz, M., Forre, O., Fu, S.-M., Natvig, J.B. & Capra, J.D. (1992) Scand.J.Immunol., 36, 349-362.
- 56 Paul, E., Iliev, A.A., Livneh, A. & Diamond, B. (1992) J.Immunol., 149, 3588-3595.
- 57 Pick, A.I., Kratzin, H.D., Barnikol-Watanabe, S. & Hilschmann, N. (1990) In Amyloid And Amyloidosis, Eds. J.B.Natvig, O.Forre, G.Husby, A.Husebekk, B.Skogen, K.Sletten & P.Westermark, Kluwer Academic
- 58 Ponstingl, H. & Hilschmann, N. (1969) Z.Physiol.Chem., 350, 1148-1152; (1971) Z.Physiol.Chem., 352, 859-877.



- 59 Ponstingl, H., Hess, M. & Hilschmann, N. (1968) *Z.Physiol.Chem.*, 349, 867-871; (1971) *Z.Physiol.Chem.*, 352, 247-266.
- 60 Randen, I., Pascual, V., Victor, K., Thompson, K.M., Forre, O., Capra, J.D. & Natvig, J.B. (1993) *Eur.J.Immunol.*, 23, 1220-1225.
- 61 Scholz, R. & Hilschmann, N. (1975) *Z.Physiol.Chem.*, 356, 1333-1335.
- 62 Settmacher, U., Jahn, S., Siegel, P., Von Baehr, R. & Hansen, A. (1993) *Mol.Immunol.*, 30, 953-954.
- 63 Shinoda, T., Titani, K. & Putnam, F.W. (1970) *J.Biol.Chem.*, 245, 4475-4487.
- 64 Sletten, K., Husby, G. & Natvig, J.B. (1974) *Scand.J.Immunol.*, 3, 833-836.; Sletten, K., Natvig, J.B., Husby, G. & Juul, J. (1981) *Biochem.J.*, 195, 561-572.
- 65 Solomon, A., Frangione, B. & Franklin, E.C. (1982) *J.Clin.Invest.*, 70, 453-460.; Frangione, B., Moloshok, T. & Solomon, A. (1983) *J.Immunol.*, 131, 2490-2493.
- 66 Takahashi, N., Takayasu, T., Isobe, T., Shinoda, T., Okuyama, T. & Shimizu, A. (1979) *J.Biochem.*, 86, 1523-1535.
- 67 Takahashi, N., Takayasu, T., Shinoda, T., Ito, S., Okuyama, T. & Shimizu, A. (1980) *Biomed.Res.*, 1, 321-333.
- 68 Takahashi, Y., Takahashi, N., Tetaert, D. & Putnam, F.W. (1983) *Proc.Nat.Acad.Sci.Usa.*, 80, 3686-3690.
- 69 Takayasu, T., Takahashi, N., Shinoda, T., Okuyama, T. & Tomioka, H. (1980) *J.Biochem.*, 89, 421-436.
- 70 Titani, K., Wikler, M., Shinoda, T. & Putnam, F.W. (1970) *J.Biol.Chem.*, 245, 2171-2176.
- 71 Toft, K.G., Sletten, K. & Husby, G. (1985) *Biol.Chem.Hoppe-Seyler*, 366, 617-625.
- 72 Tonoike, H., Kametani, F., Hoshi, A., Shinoda, T. & Isobe, T. (1985) *Biochem.Biophys.Res.Comm.*, 126, 1228-1234.
- 73 Tonoike, H., Kametani, F., Hoshi, A., Shinoda, T. & Isobe, T. (1985) *Febs Letters*, 185, 139-141.
- 74 Tsujimoto, Y. & Croce, C.M. (1984) *Nucl.Acids Res.*, 12, 8407-8414.
- 75 Tsunetsugu-Yokota, Y., Minekawa, T., Shigemoto, K., Shirasawa, T. & Takemori, T. (1992) *Mol.Immunol.*, 29, 723-728.
- 76 Tveteraas, T., Sletten, K. & Westermarck, P. (1985) *Biochem.J.*, 232, 183-190.
- 77 Vasicek, T.J. & Leder, P. (1990) *J.Exp.Med.*, 172, 609-620.

- 78 Victor, K.D., Randen, I., Thompson, K., Forre, O., Natvig, J.B., Fu, S.M. & Capra, J.D. (1991) J.Clin.Invest., 87, 1603-1613.
- 79 Weng, N.-P., Yu-Lee, L.-Y., Sanz, I., Patten, B.M. & Marcus, D.M. (1992) J.Immunol., 149, 2518-2529.
- 80 Wikler, M. & Putnam, F.W. (1970) J.Biol.Chem., 245, 4488-4507.
- 81 Winkler, T.H., Fehr, H. & Kalden, J.R. (1992) Eur.J.Immunol., 22, 1719-1728.
- 82 Yago, K., Zenita, K., Ohwaki, I., Harada, Y., Nozawa, S., Tsukazaki, K., Iwamori, M., Endo, N., Yasuda, N., Okuma, M. & Kannagi, R. (1993) Mol.Immunol., 30, 1481-1489.
- 83 Yamasaki, N., Komori, S. & Watanabe, T. (1987) Mol.Immunol., 24, 981-985.
- 84 Zhu, D., Kim, H.S. & Deutsch, H.F. (1983) Mol.Immunol., 20, 1107-1116.
- 85 Zhu, D., Zhang, H., Zhu, N. & Luo, X. (1986) Scientia Sinica, 29, 746-755.

References of rearranged human heavy chain sequences used for alignment

- 1 Adderson, E.E., Azmi, F.H., Wilson, P.M., Shackelford, P.G. & Carroll, W.L. (1993) J.Immunol., 151, 800-809.
- 2 Adderson, E.E., Shackelford, P.G., Quinn, A. & Carroll, W.L. (1991) J.Immunol., 147, 1667-1674.
- 3 Akahori, Y., Kurosawa, Y., Kamachi, Y., Torii, S. & Matsuoka, H. (1990) J.Clin.Invest., 85, 1722-1727.
- 4 Andris, J.S., Brodeur, B.R. & Capra, J.D. (1993) Mol.Immunol., 30, 1601-1616.
- 5 Andris, J.S., Ehrlich, P.H., Ostberg, L. & Capra, J.D. (1992) J.Immunol., 149, 4053-4059.
- 6 Andris, J.S., Johnson, S., Zolla-Pazner, S. & Capra, J.D. (1991) Proc.Natl.Acad.Sci.Usa, 88, 7783-7787.
- 7 Anker, R., Conley, M.E. & Pollok, B.A. (1989) J.Exp.Med., 169, 2109-2119.
- 8 Atkinson, P.M., Lampman, G.W., Furie, B.C., Naparstek, Y., Schwartz, R.S., Stollar, B.D. & Furie, B. (1985) J.Clin.Invest., 75, 1138-1143.; Lampman, G.W., Furie, B., Schwartz, R.S., Stollar, B.D. & Furie, B.C. (1989)
- 9 Avila, M.A., Vazques, J., Danielsson, L., Fernández De Cossio, M.E. & Borrebaeck, C.A.K. (1993) Gene, 127, 273-274.
- 10 Bakkus, M.H.C., Heirman, C., Van Riet, I., Van Camp, B. & Thielemans, K. (1992) Blood, 80, 2326-2335.

- 11 Barbas Iii, C.F., Crowe, Jr., J.E., Cababa, D., Jones, T.M., Zebedee, S.L., Murphy, B.R., Chanock, R.M. & Burton, D.R. (1992) *Proc.Natl.Acad.Sci.Usa*, 89, 10164-10168.
- 12 Barbas, C.F., Iii, Collet, T.A., Amberg, W., Roben, P., Binley, J.M., Hoekstra, D., Cababa, D., Jones, T.M., Williamson, R.A., Pilkington, G.R., Haigwood, N.L., Cabezas, E., Satterthwait, A.C., Sanz, I. & Burton, D.R. (1993) *J.Mol.Biol.*, 230, 812-823.
- 13 Berman, J.E., Humphries, C.G., Barth, J., Alt, F.W. & Tucker, P.W. (1991) *J.Exp.Med.*, 173, 1529-1535.
- 14 Berman, J.E., Mellis, S.J., Pollock, R., Smith, C.L., Suh, H., Heinke, B., Kowal, C., Surti, U., Chess, L., Cantor, C.R & Alt, F.W. (1988) *Embo J.*, 7, 727-738.
- 15 Bhat, N.M., Bieber, M.M., Chapman, C.J., Stevenson, F.K. & Teng, N.N.H. (1993) *J.Immunol.*, 151, 5011-5021.
- 16 Bird, J., Galili, N., Link, M., Stites, D. & Sklar, J. (1988) *J.Exp.Med.*, 168, 229-245.
- 17 Cai, J., Humphries, C., Richardson, A. & Tucker, P.W. (1992) *J.Exp.Med.*, 176, 1073-1081.
- 18 Cairns, E., Kwong, P.C., Misener, V., Ip, P., Bell, D.A. & Siminovitch, K.A. (1989) *J.Immunol.*, 143, 685-691.
- 19 Capra, J.D. & Hopper, J.E. (1976) *Immunochemistry*, 13, 995-999; Hopper, J.E., Noyes, C., Heinrichson, R. & Kessel, J.W. (1976) *J.Immunol.*, 116, 743-746.
- 20 Capra, J.D. & Kehoe, J.M. (1974) *Proc.Nat.Acad.Sci.Usa*, 71, 845-848.
- 21 Carroll, W.L., Yu, M., Link, M.P. & Korsmeyer, S.J. (1989) *J.Immunol.*, 143, 692-698.
- 22 Chen, P.P., Liu, M.-F., Glass, C.A., Sinha, S., Kipps, T.J. & Carson, D.A. (1989) *Arthritis & Rheumatism*, 32, 72-76; Kipps, T.J., Tomhave, E., Pratt, L.F., Duffy, S., Chen, P.P. & Carson, D.A. (1989) *Proc.Natl.Acad.Sci.Usa*, 86, 5913-5917.
- 23 Chiu, Y.Y.H., Lopez De Castro, J.A. & Poljak, R.J. (1979) *Biochemistry*, 18, 553-560.
- 24 Cleary, M.L., Meeker, T.C., Levy, S., Lee, E., Trela, M., Sklar, J. & Levy, R. (1986) *Cell*, 44, 97-106.
- 25 Cuisinier, A.-M., Fumoux, F., Fougereau, M. & Tonnelle, C. (1992) *Mol.Immunol.*, 29, 1363-1373.
- 26 Cuisinier, A.-M., Gauthier, L., Boubli, L., Fougereau, M. & Tonnelle, C. (1993) *Eur.J.Immunol.*, 23, 110-118.
- 27 Cunningham, B.A., Gottlieb, P.D., Pflumm, M.N. & Edelman, G.M. (1971) *Progress In Immunology* (B.Amos, Ed.), Academic Press, N.Y., Pp.3-24.

- 28 Cunningham, B.A., Rutishauser, U., Gall, W.E., Gottlieb, P.D., Waxdal, M.J. & Edelman, G.M. (1970) *Biochemistry*, 9, 3161-3170.
- 29 Deane, M. & Norton, J.D. (1990) *Eur.J.Immunol.*, 20, 2209-2217.
- 30 Deane, M. & Norton, J.D. (1991) *Leukemia*, 5, 646-650.
- 31 Dersimonian, H., Schwartz, R.S., Barrett, K.J. & Stollar, B.D. (1987) *J.Immunol.*, 139, 2496-2501.
- 32 Dersimonian, H., Schwartz, R.S., Barrett, K.J. & Stollar, B.D. (1987) *J.Immunol.*, 139, 2496-2501; Chen, P.P., Liu, M.-F., Sinha, S. & Carson, D.A. (1988) *Arth.Rheum.*, 31, 1429-1431.
- 33 Desai, R., Spatz, L., Matsuda, T., Ilyas, A.A., Berman, J.E., Alt, F.W., Kabat, E.A. & Latov, N. (1990) *J.Neuroimmunol.*, 26, 35-41.
- 34 Ezaki, I., Kanda, H., Sakai, K., Fukui, N., Shingu, M., Nobunaga, M. & Watanabe, T. (1991) *Arthritis And Rheumatism*, 34, 343-350.
- 35 Felgenhauer, M., Kohl, J. & Ruker, F. (1990) *Nucl.Acids Res.*, 18, 4927.
- 36 Florent, G., Lehman, D. & Putnam, F.W. (1974) *Biochemistry*, 13, 2482-2498.
- 37 Friedlander, R.M., Nussenzweig, M.C. & Leder, P. (1990) *Nucl.Acids Res.*, 18, 4278.
- 38 Gawinowicz, M.A., Merlini, G., Birken, S., Osserman, E.F. & Kabat, E.A. (1991) *J.Immunol.*, 147, 915-920.
- 39 Gillies, S.D., Dorai, H., Wesolowski, J., Majeau, G., Young, D., Boyd, J., Gardner, J. & James, K. (1989) *Bio/Tech.*, 7, 799-804.
- 40 Goni, F. & Frangione, B. (1983) *Proc.Nat.Acad.Sci.Usa*, 80, 4837-4841.
- 41 Gorman, S.D., Clark, M.R., Routledge, E.G., Cobbold, S.P. & Waldmann, H. (1991) *Proc.Natl.Acad.Sci.Usa*, 88, 4181-4185.
- 42 Griffiths, A.D., Malmqvist, M., Marks, J.D., Bye, J.M., Embleton, M.J., Mccafferty, J., Baier, M., Holliger, K.P., Gorick, B.D., Hughes-Jones, N.C., Hoogenboom, H.R. & Winter, G. (1993) *Embo J.*, 12, 725-734.
- 43 Grillot-Courvalin, C., Brouet, J.-C., Piller, F., Rassenti, L.Z., Labaume, S., Silverman, G.J., Silberstein, L. & Kipps, T.J. (1992) *Eur.J.Immunol.*, 22, 1781-1788.
- 44 Guillaume, T., Rubinstein, D.B., Young, F., Tucker, L., Logtenberg, T., Schwartz, R.S. & Barrett, K.L. (1990) *J.Immunol.*, 145, 1934-1945; Young, F., Tucker, L., Rubinstein, D., Guillaume, T., Andre-Schwartz, J., Barrett, K.J., Schwartz, R.S. & Logtenberg, T. (1990)
- 45 Harindranath, N., Goldfarb, I.S., Ikematsu, H., Burastero, S.E., Wilder, R.L., Notkins, A.L. & Casali, P. (1991) *Int.Immunol.*, 3, 865-875.

- 46 Hillson, J.L., Oppliger, I.R., Sasso, E.H., Milner, E.C.B. & Wener, M.H. (1992) *J.Immunol.*, 149, 3741-3752.
- 47 Hirabayashi, Y., Munakata, Y., Sasaki, T. & Sano, H. (1992) *Nucl.Acids Res.*, 20, 2601.
- 48 Hoch, S. & Schwaber, J. (1987) *J.Immunol.*, 139, 1689-1693.
- 49 Huang, C., Stewart, A.K., Schwartz, R.S. & Stollar, B.D. (1992) *J.Clin.Invest.*, 89, 1331-1343.
- 50 Hughes-Jones, N.C., Bye, J.M., Beale, D. & Coadwell, J. (1990) *Biochem.J.*, 268, 135-140.
- 51 Ikematsu, H., Harindranath, N., Ueki, Y., Notkins, A.L. & Casali, P. (1993) *J.Immunol.*, 150, 1325-1337.
- 52 Ikematsu, H., Kasaian, M.T., Schettino, E.W. & Casali, P. (1993) *J.Immunol.*, 151, 3604-3616.
- 53 Kelly, P.J., Pascual, V., Capra, J.D. & Lipsky, P.E. (1992) *J.Immunol.*, 148, 1294-1301.
- 54 Kipps, T.J. & Duffy, S.F. (1991) *J.Clin.Invest.*, 87, 2087-2096.
- 55 Kipps, T.J., Tomhave, E., Pratt, L.F., Duffy, S., Chen, P.P. & Carson, D.A. (1989) *Proc.Natl.Acad.Sci.Usa*, 86, 5913-5917.
- 56 Kishimoto, T., Okajima, H., Okumoto, T. & Taniguchi, M. (1989) *Nucl.Acids Res.*, 17, 4385.
- 57 Knight, G.B., Agnello, V., Bonagura, V., Barnes, J.L., Panka, D.J. & Zhang, Q.-X. (1993) *J.Exp.Med.*, 178, 1903-1911.
- 58 Kohler, H., Shimizu, A., Paul, C., Moore, V. & Putnam, F.W. (1970) *Nature*, 227, 1318-1320; Florent, G., Lehman, D. & Putnam, F.W. (1974) *Biochemistry*, 13, 2482-2498
- 59 Komori, S., Yamasaki, N., Shigeta, M., Isojima, S. & Watanabe, T. (1988) *Clin.Exp.Immunol.*, 71, 508-516.
- 60 Kon, S., Levy, S. & Levy, R. (1987) *Proc.Natl.Acad.Sci.Usa*, 84, 5053-5057.
- 61 Kratzin, H., Altevogt, P., Ruban, E., Kortt, A., Staroscik, K. & Hilschmann, N. (1975) *Z.Physiol.Chem.*, 356, 1337-1342; Kratzin, H., Altevogt, P., Kortt, A., Ruban, E. & Hilschmann, N. (1978) *Z.Physiol.Chem.*, 359, 1717-1745.
- 62 Kudo, A., Ishihara, T., Nishimura, Y. & Watanabe, T. (1985) *Gene*, 33, 181-189.
- 63 Kunicki, T.J., Annis, D.S., Gorski, J. & Nugent, D.J. (1991) *J.Autoimmunity*, 4, 433-446.
- 64 Larrick, J.W., Wallace, E.F., Coloma, M.J., Bruderer, U., Lang, A.B. & Fry, K.E. (1992) *Immunological Reviews*, 130, 69-85.
- 65 Lehman, D.W. & Putnam, F.W. (1980) *Proc.Natl.Acad.Sci.Usa*, 77, 3239-3243.

- 66 Lewis, A.P., Lemon, S.M., Barber, K.A., Murphy, P., Parry, N.R., Peakman, T.C., Sims, M.J., Worden, J. & Crowe, J.S. (1993) *J.Immunol.*, 151, 2829-2838.
- 67 Liu, V.Y.S., Low, T.L.K., Infante, A. & Putnam, F.W. (1976) *Science*, 193, 1017-1020.
- 68 Logtenberg, T., Young, F.M., Van Es, J., Gmelig-Meyling, F.H.J., Berman, J.E. & Alt, F.W. (1989) *J.Autoimmunity*, 2, 203-213.
- 69 Logtenberg, T., Young, F.M., Van Es, J.H., Gmelig-Meyling, F.H.J. & Alt, F.W. (1989) *J.Exp.Med.*, 170, 1347-1355.
- 70 Manheimer-Lory, A., Katz, J.B., Pillinger, M., Ghossein, C., Smith, A. & Diamond, B. (1991) *J.Exp.Med.*, 174, 1639-1652.
- 71 Mantovani, L., Wilder, R.L. & Casali, P. (1993) *J.Immunol.*, 151, 473-488.
- 72 Mariette, X., Tsapis, A. & Brouet, J.-C. (1993) *Eur.J.Immunol.*, 23, 846-851.
- 73 Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., Mccafferty, J., Griffiths, A.D. & Winter, G. (1991) *J.Mol.Biol.*, 222, 581-597.
- 74 Meeker, T.C., Grimaldi, J., O'rourke, R., Loeb, J.Juliusson, G. & Einhorn, S. (1988) *J.Immol.*, 141, 3994-3998.
- 75 Milili, M., Fougereau, M., Guglielmi, P. & Schiff, C. (1991) *Mol.Immunol.*, 28, 753-761.
- 76 Moran, M.J., Andris, J.S., Matsumoto, Y.-I., Capra, J.D. & Hersh, E.M. (1993) *Mol.Immunol.*, 30, 1543-1551.
- 77 Mortari, F., Wang, J.-Y. & Schroeder, Jr., H.W. (1993) *J.Immunol.*, 150, 1348-1357.
- 78 Newkirk, M.M., Gram, H., Heinrich, G.F., Ostberg, L., Capra, J.D. & Wasserman, R.L (1988) *J.Clin.Invest.*, 81, 1511-1518.
- 79 Newkirk, M.M., Mageed, R.A., Jefferis, R., Chen, P.P. & Capra, J.D. (1987) *J.Exp.Med.*, 166, 550-564.
- 80 Nickerson, K.G., Berman, J., Glickman, E., Chess, L. & Alt, F.W. (1989) *J.Exp.Med.*, 169, 1391-1403.
- 81 Olee, B.T., Lu, E.W., Huang, D.-F., Soto-Gil, R.W., Deftos, M., Kozin, F., Carson, D.A. & Chen, P.P. (1992) *J.Exp.Med.*, 175, 831-842.
- 82 Pascual, V., Randen, I., Thompson, K., Sioud, M.Forre, O., Natvig, J. & Capra, J.D. (1990) *J.Clin.Invest.*, 86, 1320-1328.
- 83 Pascual, V., Randen, I., Thompson, K., Sioud, M.Forre, O., Natvig, J. & Capra, J.D. (1990) *J.Clin.Invest.*, 86, 1320-1328; Randen, I., Brown, D., Thompson, K.M., Hughes-Jones, N., Pascual, V., Victor, K., Capra, J.D., Forre, O. & Natvig, J.B. (1992)

- 84 Pascual, V., Victor, K., Lelsz, D., Spellerberg, M.B., Hamblin, T.J., Thompson, K.M., Randen, I., Natvig, J., Capra, J.D. & Stevenson, F.K. (1991) *J.Immunol.*, 146, 4385-4391.
- 85 Pascual, V., Victor, K., Randen, I., Thompson, K., Steinitz, M., Forre, O., Fu, S.-M., Natvig, J.B. & Capra, J.D. (1992) *Scand.J.Immunol.*, 36, 349-362.
- 86 Pascual, V., Victor, K., Spellerberg, M., Hamblin, T.J., Stevenson, F.K. & Capra, J.D. (1992) *J.Immunol.*, 149, 2337-2344.
- 87 Ponstingl, H., Schwarz, J., Reichel, W. & Hilschmann, N. (1970) *Z.Physiol.Chem.*, 351, 1591-1594.; Ponstingl, H. & Hilschmann, N. (1976) *Z.Physiol.Chem.*, 357, 1571-1604.
- 88 Portolano, S., Mclachlan, S.M. & Rapoport, B. (1993) *J.Immunol.*, 151, 2839-2851.
- 89 Portolano, S., Seto, P., Chazenbalk, G.D., Nagayama, Y., Mclachlan, S.M. & Rapoport, B. (1991) *Biochem.Biophys.Res.Comm.*, 179, 372-377.
- 90 Pratt, L.F., Szubin, R., Carson, D.A. & Kipps, T.J. (1991) *J.Immunol.*, 147, 2041-2046.
- 91 Press, E.M. & Hogg, N.M. (1970) *Biochem.J.*, 117, 641-660.
- 92 Putnam, F.W., Shimizu, A., Paul, C., Shinoda, T. & Kohler, H. (1971) *Ann.N.Y.Acad.Sci.*, 190, 83-103.
- 93 Putnam, F.W., Takahashi, N., Tetaert, D., Debuire, B. & Lin, L.C. (1981) *Proc.Nat.Acad.Sci.Usa*, 78, 6168-6172.; Takahashi, N., Tetaert, D., Debuire, B., Lin, L. & Putnam, F.W. (1982) *Proc.Nat.Acad.Sci.Usa*, 79, 2850-2854.
- 94 Raaphorst, F.M., Timmers, E., Kenter, M.J.H., Van Tol, M.J.D., Vossen, J.M. & Schuurman, R.K.B. (1992) *Eur.J.Immunol.*, 22, 247-251.
- 95 Rabbitts, T.H., Bentley, D.L., Dunnick, W., Forster, A., Matthyssens, G. & Milstein, C. (1980) *Cold Spring Harb.Symp.Quanti.Biol.*, 45, 867-878; Matthyssens, G. & Rabbitts, T.H. (1980) *Proc.Nat.Acad.Sci.Usa*, 77, 6561-6565.
- 96 Randen, I., Pascual, V., Victor, K., Thompson, K.M., Forre, O., Capra, J.D. & Natvig, J.B. (1993) *Eur.J.Immunol.*, 23, 1220-1225.
- 97 Rassenti, L.Z. & Kipps, T.J. (1993) *J.Exp.Med.*, 177, 1039-1046.
- 98 Reidl, L.S., Friedman, D.F., Goldman, J., Hardy, R.R., Jefferies, L.C. & Silberstein, L.E. (1991) *J.Immunol.*, 147, 3623-3631.
- 99 Roudier, J., Silverman, G.J., Chen, P.P., Carson, D.A. & Kipps, T.J. (1990) *J.Immunol.*, 144, 1526-1530.
- 100 Sanz, I., Casali, P., Thomas, J.W., Notkins, A.L. & Capra, J.D. (1989) *J.Immunol.*, 142, 4054-4061.

- 101 Sanz, I., Dang, H., Takei, M., Talal, N. & Capra, J.D. (1989) *J.Immunol.*, 142, 883-887.
- 102 Schmidt, W.E., Jung, H.-D., Palm, W. & Hilschmann, N. (1983) *Z.Physiol.Chem.*, 364, 713-747.
- 103 Schroeder, H.W., Jr. & Wang, J.Y. (1990) *Proc.Natl.Acad.Sci.Usa*, 87, 6146-6150.
- 104 Schroeder, H.W., Jr., Hillson, J.L. & Perlmutter, R.M. (1987) *Science*, 238, 791-793.
- 105 Schroeder, H.W., Jr., Hillson, J.L. & Perlmutter, R.M. (1987) *Science*, 238, 791-793; Chen, P.P., Liu, M.-F., Glass, C.A., Sinha, S., Kipps, T.J. & Carson, D.A. (1989) *Arthritis & Rheumatism*, 32, 72-76.
- 106 Schroeder, H.W., Jr., Hillson, J.L. & Perlmutter, R.M. (1987) *Science*, 238, 791-793; Chen, P.P., Liu, M.-F., Sinha, S. & Carson, D.A. (1988) *Arth.Rheum.*, 31, 1429-1431.
- 107 Schutte, M.E., Ebeling, S.B., Akkermans, K.E., Gmelig-Meyling, F.H. & Logtenberg, T. (1991) *Eur.J.Immunol.*, 21, 1115-1121.
- 108 Schutte, M.E., Ebeling, S.B., Akkermans, K.E., Gmelig-Meyling, F.H.J. & Logtenberg, T. (1991) *Eur.J.Immunol.*, 21, 1115-1121.
- 109 Settmacher, U., Jahn, S., Siegel, P., Von Baehr, R. & Hansen, A. (1993) *Mol.Immunol.*, 30, 953-954.
- 110 Shen, A., Humphries, C., Tucker, P. & Blattner, F. (1987) *Proc.Natl.Acad.Sci.Usa*, 84, 8563-8567.
- 111 Shimizu, A., Nussenzweig, M.C., Mizuta, T.-R., Leder, P. & Honjo, T. (1989) *Proc.Natl.Acad.Sci.Usa*, 86, 8020-8023.
- 112 Shin, E.K., Matsuda, F., Fujikura, J., Akamizu, T., Sugawa, H., Mori, T. & Honjo, T. (1993) *Eur.J.Immunol.*, 23, 2365-2367.
- 113 Silberstein, L.E., Litwin, S. & Carmack, C.E. (1989) *J.Exp.Med.*, 169, 1631-1643.
- 114 Singal, D.P., Frame, B., Joseph, S., Blajchman, M.A. & Leber, B.F. (1993) *Immunogenet.*, 38, 242.
- 115 Spatz, L.A., Wong, K.K., Williams, M., Desai, R., Golier, J., Berman, J.E., Alt, F.W. & Latov, N. (1990) *J.Immunol.*, 144, 2821-2828.
- 116 Steiner, L.A., Garcia-Pardo, A. & Margolies, M.N. (1979) *Biochemistry*, 18, 4068-4080.
- 117 Stewart, A.K., Huang, C., Stollar, B.D. & Schwartz, R.S. (1993) *J.Exp.Med.*, 177, 409-418.
- 118 Thomas, J.W. (1993) *J.Immunol.*, 150, 1375-1382.
- 119 Torano, A. & Putnam, F.W. (1978) *Proc.Nat.Acad.Sci.Usa*, 75, 966-969.



- 120 Van Der Heijden, R.W.J., Bunschoten, H., Pascual, V., Uytdehaag, F.G.C.M., Osterhaus, A.D.M.E. & Capra, J.D. (1990) J.Immunol., 144, 2835-2839.
- 121 Van Der Stoep, N., Van Der Linden, J. & Logtenberg, T. (1993) J.Exp.Med., 177, 99-107.
- 122 Van Es, J.H., Gmelig-Meyling, F.H.J. & Logtenberg, T. (1992) Eur.J.Immunol., 22, 2761-2764.
- 123 Varade, W.S., Marin, E., Kittelberger, A.M. & Insel, R.A. (1993) J.Immunol., 150, 4985-4995.
- 124 Victor, K.D., Pascual, V., Lefvert, A.K. & Capra, J.D. (1992) Mol.Immunol., 29, 1501-1506.
- 125 Victor, K.D., Pascual, V., Williams, C.L., Lennon, V.A. & Capra, J.D. (1992) Eur.J.Immunol., 22, 2231-2236.
- 126 Watanabe, S., Barnikol, H.U., Horn, J., Bertram, J. & Hilschmann, N. (1973) Z.Physiol.Chem., 354, 1505-1509.
- 127 Weng, N.-P., Yu-Lee, L.-Y., Sanz, I., Patten, B.M. & Marcus, D.M. (1992) J.Immunol., 149, 2518-2529.
- 128 White, M.B., Word, C.J., Humphries, C.G., Blattner, F.R. & Tucker, P.W. (1990) Mol.Cell.Biol., 10, 3690-3699.
- 129 Winkler, T.H., Fehr, H. & Kalden, J.R. (1992) Eur.J.Immunol., 22, 1719-1728.
- 130 Yago, K., Zenita, K., Ohwaki, I., Harada, Y., Nozawa, S., Tsukazaki, K., Iwamori, M., Endo, N., Yasuda, N., Okuma, M. & Kannagi, R. (1993) Mol.Immunol., 30, 1481-1489.
- 131 Zelenetz, A.D., Chen, T.T. & Levy, R. (1992) J.Exp.Med., 176, 1137-1148.

*B. References of germline sequences*

References of human germline kappa sequences

- 1 Cox, J.P.L., Tomlinson, I.M. & Winter, G. (1994) Eur.J.Immunol., 24, 827-836.
- 2 Huber, C., Et Al. (1993) Eur.J.Immunol., 23, 2868.
- 3 Klobeck, H.G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.D. & Zachau, H.G. (1985) Nucl.Acids Res., 13, 6515-6529.
- 4 Lautner-Rieske, A., Huber, C., Meindl, A., Pargent, W., Schäble, K.F., Thiebe, R., Zocher, I. & Zachau, H.G. (1992) Eur.J.Immunol. 22, 1023.
- 5 Lorenz, W., Schäble, K.F., Thiebe, R., Stavnezer, J. & Zachau, H.G. (1988) Mol.Immunol., 25, 479.

- 6 Pargent, W., Meindl, A., Thiebe, R., Mitzel, S. & Zachau, H.G. (1991) Eur.J.Immunol., 21, 1821-1827.
- 7 Pech, M. & Zachau, H.G. (1984) Nuc.Acids Res., 12, 9229-9236.
- 8 Pech, M., Jaenichen, H.-R., Pohlenz, H.-D., Neumaier, P.S., Klobeck, H.-G. & Zachau, H.G. (1984) J.Mol.Biol., 176, 189-204.
- 9 Scott, M.G., Crimmins, D.L., Mccourt, D.W., Chung, G., Schable, K.F., Thiebe, R., Quenzel, E.-M., Zachau, H.G. & Nahm, M.H. (1991) J.Immunol., 147, 4007-4013.
- 10 Stavnezer, J., Kekish, O., Batter, D., Grenier, J., Balazs, I., Henderson, E. & Zegers, B.J.M. (1985) Nucl.Acids Res., 13, 3495-3514.
- 11 Straubinger, B., Huber, E., Lorenz, W., Osterholzer, E., Pargent, W., Pech, M., Pohlenz, H.-D., Zimmer, F.-J. & Zachau, H.G. (1988) J.Mol.Biol., 199, 23-34.
- 12 Straubinger, B., Thiebe, R., Huber, C., Osterholzer, E. & Zachau, H.G. (1988) Biol.Chem.Hoppe-Seyer, 369, 601-607.

References of human germline lambda sequences

- 1 Williams, S.C. & Winter, G. (1993) Eur.J.Immunol., 23, 1456-1461.
- 2 Siminovitch, K.A., Misener, V., Kwong, P.C., Song, Q.-L. & Chen, P.P. (1989) J.Clin.Invest., 84, 1675-1678.
- 3 Brockly, F., Alexandre, D., Chuchana, P., Huck, S., Lefranc, G. & Lefranc, M.-P. (1989) Nuc.Acids.Res., 17, 3976.
- 4 Daley, M.D., Peng, H.-Q., Misener, V., Liu, X.-Y., Chen, P.P. & Siminovitch, K.A. (1992) Mol.Immunol., 29, 1515-1518.
- 5 Deftos, M., Soto-Gil, R., Quan, M., Olee, T. & Chen, P.P. (1994) Scand. J. Immunol., 39, 95.
- 6 Stiernholm, N.B.J., Kuzniar, B. & Berinstein, N.L. (1994) J. Immunol., 152, 4969-4975.
- 7 Combriato, G. & Klobeck, H.G. (1991) Eur.J.Immunol., 21, 1513-1522.
- 8 Anderson, M.L.M., Szajnert, M.F., Kaplan, J.C., Mccoll, L. & Young, B.D. (1984) Nuc.Acids Res., 12, 6647-6661.

References of human germline heavy chain sequences

- 1 Adderson, E.E., Azmi, F.H., Wilson, P.M., Shackelford, P.G. & Carroll, W.L. (1993) J.Immunol., 151, 800-809.
- 2 Andris, J.S., Brodeur, B.R. & Capra, J.D. (1993) Mol.Immunol., 30, 1601-1616.

- 3 Berman, J.E., Mellis, S.J., Pollock, R., Smith, C.L., Suh, H., Heinke, B., Kowal, C., Surti, U., Chess, L., Cantor, C.R. & Alt, F.W. (1988) *Embo J.*, 7, 727-738.
- 4 Buluwela, L. & Rabbitts, T.H. (1988) *Eur.J.Immunol.*, 18, 1843-1845.; Buluwela, L., Albertson, D.G., Sherrington, P., Rabbitts, P.H., Spurr, N. & Rabbitts, T.H. (1988) *Embo J.*, 7, 2003-2010.
- 5 Chen, P.P., Liu, M.-F., Sinha, S. & Carson, D.A. (1988) *Arth.Rheum.*, 31, 1429-1431.
- 6 Chen, P.P., Liu, M.-F., Glass, C.A., Sinha, S., Kipps, T.J. & Carson, D.A. (1989) *Arthritis & Rheumatism*, 32, 72-76.
- 7 Cook, G.P. et al. (1994) *Nature Genetics* 7, 162-168.
- 8 Haino, M. et al., (1994). *J. Biol. Chem.* 269, 2619-2626
- 9 Humphries, C.G., Shen, A., Kuziel, W.A., Capra, J.D., Blattner, F.R. & Tucker, P.W. (1988) *Nature*, 331, 446-449.
- 10 Kodaira, M., Kinashi, T., Umemura, I., Matsuda, F., Noma, T., Ono, Y. & Honjo, T. (1986) *J.Mol.Biol.*, 190, 529-541.
- 11 Lee, K.H., Matsuda, F., Kinashi, T., Kodaira, M. & Honjo, T. (1987) *J.Mol.Biol.*, 195, 761-768.
- 12 Matsuda, F., Lee, K.H., Nakai, S., Sato, T., Kodaira, M., Zong, S.Q., Ohno, H., Fukuhara, S. & Honjo, T. (1988) *Embo J.*, 7, 1047-1051.
- 13 Matsuda, F., Shin, E.K., Hirabayashi, Y., Nagaoka, H., Yoshida, M.C., Zong, S.Q. & Honjo, T. (1990) *Embo J.*, 9, 2501-2506.
- 14 Matsuda, F., Shin, E.K., Nagaoka, H., Matsumura, R., Haino, M., Fukita, Y., Taka-Ishi, S., Imai, T., Riley, J.H., Anand, R. Et, Al. (1993) *Nature Genet.* 3, 88-94
- 15 Nagaoka, H., Ozawa, K., Matsuda, F., Hayashida, H., Matsumura, R., Haino, M., Shin, E.K., Fukita, Y., Imai, T., Anand, R., Yokoyama, K., Eki, T., Soeda, E. & Honjo, T. (1993).  
(Temporal)
- 16 Rechavi, G., Bienz, B., Ram, D., Ben-Neriah, Y., Cohen, J.B., Zakut, R. & Givol, D. (1982) *Proc.Nat.Acad.Sci.Usa*, 79, 4405-4409.
- 17 Sanz, I., Kelly, P., Williams, C., Scholl, S., Tucker, P. & Capra, J.D. (1989) *Embo J.*, 8, 3741-3748.
- 18 Shin, E.K., Matsuda, F., Fujikura, J., Akamizu, T., Sugawa, H., Mori, T. & Honjo, T. (1993) *Eur.J.Immunol.*, 23, 2365-2367.
- 19 Tomlinson, Im., Walter, G., Marks, Jd., Llewelyn, Mb. & Winter, G. (1992) *J.Mol.Biol.* 227, 776-798.

- 20 Van Der Maarel, S., Van Dijk, K.W., Alexander, C.M., Sasso, E.H., Bull, A. & Milner, E.C.B. (1993) J.Immunol., 150, 2858-2868.
- 21 Van Dijk, K.W., Mortari, F., Kirkham, P.M., Schroeder, Jr., H.W. & Milner, E.C.B. (1993) Eur.J.Immunol., 23, 832-839.
- 22 Van Es, J.H., Aanstoot, H., Gmelig-Meyling, F.H.J., Derksen, R.H.W.M. & Logtenberg, T. (1992) J.Immunol., 149, 2234-2240.
- 23 Weng, N.-P., Snyder, J.G., Yu-Lee, L.-Y. & Marcus, D.M. (1992) Eur.J.Immunol., 22, 1075-1082.
- 24 Winkler, T.H., Fehr, H. & Kalden, J.R. (1992) Eur.J.Immunol., 22, 1719-1728.
- 25 Olee, T., Yang, P.M., Siminovitch, K.A., Olsen, N.J., Hillson, J.L., Wu, J., Kozin, F., Carson, D.A.&Chen, P.P. (1991) J. Clin. Invest. 88, 193-203.
- 26 Chen, P.P.& Yang, P.M. (1990) Scand. J. Immunol. 31, 593-599.
- 27 Tomlinson, M., Walter, G., Cook&Winter, G. (Unpublished)

## Claims

1. A method of setting up one or more nucleic acid sequences encoding one or more (poly)peptide sequences suitable for the creation of libraries of (poly)peptides said (poly)peptide sequences comprising amino acid consensus sequences, said method comprising the following steps:
  - (a) deducing from a collection of at least three homologous proteins one or more (poly)peptide sequences comprising at least one amino acid consensus sequence;
  - (b) optionally, identifying amino acids in said (poly)peptide sequences to be modified so as to remove unfavorable interactions between amino acids within or between said or other (poly)peptide sequences;
  - (c) identifying at least one structural sub-element within each of said (poly)peptide sequences;
  - (d) backtranslating each of said (poly)peptide sequences into a corresponding coding nucleic acid sequence;
  - (e) setting up cleavage sites in regions adjacent to or between the ends of sub-sequences encoding said sub-elements, each of said cleavage sites:
    - (ea) being unique within each of said coding nucleic acid sequences;
    - (eb) being common to the corresponding sub-sequences of any said coding nucleic acids.
2. A method of setting up two or more sets of one or more nucleic acid sequences comprising executing the steps described in claim 1 for each of said sets with the additional provision that said cleavage sites are unique between said sets.
3. The method of claim 2 in which at least two of said sets are deduced from the same collection of at least three homologous proteins.
4. The method according to any one of claims 1 to 3, wherein said setting up further comprises the synthesis of said nucleic acid coding sequences.
5. The method according to any one of claims 1 to 4, further comprising the cloning of said nucleic acid coding sequences into a vector.

6. The method according to any one of claims 1 to 5, wherein said removal of unfavorable interactions results in enhanced expression of said (poly)peptides.
7. The method according to any one of claims 1 to 6, further comprising the steps of:
  - (f) cleaving at least two of said cleavage sites located in regions adjacent to or between the ends of said sub-sequences; and
  - (g) exchanging said sub-sequences by different sequences; and
  - (h) optionally, repeating steps (f) and (g) one or more times.
8. The method according to claim 7, wherein said different sequences are selected from the group of different sub-sequences encoding the same or different sub-elements derived from the same or different (poly)peptides.
9. The method according to claims 7 or 8, wherein said different sequences are selected from the group of:
  - (i) genomic sequences or sequences derived from genomic sequences;
  - (ii) rearranged genomic sequences or sequences derived from rearranged genomic sequences; and
  - (iii) random sequences.
10. The method according to any one of claims 1 to 9 further comprising the expression of said nucleic acid coding sequences.
11. The method according to any one of claims 1 to 10 further comprising the steps of:
  - (i) screening, after expression, the resultant (poly)peptides for a desired property;
  - (k) optionally, repeating steps (f) to (i) one or more times with nucleic acid sequences encoding one or more (poly)peptides obtained in step (i).
12. The method according to claim 11, wherein said desired property is selected from the group of optimized affinity or specificity for a target molecule, optimized enzymatic activity, optimized expression yields, optimized stability and optimized solubility.

13. The method according to any one of claims 1 to 12, wherein said cleavage sites are sites cleaved by restriction enzymes.
14. The method according to any one of claims 1 to 13, wherein said structural sub-elements comprise between 1 and 150 amino acids.
15. The method according to claim 14, wherein said structural sub-elements comprise between 3 and 25 amino acids.
16. The method according to any one of claims 1 to 15, wherein said nucleic acid is DNA.
17. The method according to any one of claims 1 to 16, wherein said (poly)peptides have an amino acid pattern characteristic of a particular species.
18. The method according to claim 17, wherein said species is human.
19. The method according to any one of claims 1 to 18, wherein said (poly)peptides are at least part of members or derivatives of the immunoglobulin superfamily.
20. The method according to claim 19, wherein said members or derivatives of the immunoglobulin superfamily are members or derivatives of the immunoglobulin family.
21. The method according to claim 19 or 20, wherein said (poly)peptides are or are derived from heavy or light chain variable regions wherein said structural sub-elements are framework regions (FR) 1, 2, 3, or 4 or complementary determining regions (CDR) 1, 2, or 3.
22. The method according to claim 20 or 21, wherein said (poly)peptides are or are derived from the HuCAL consensus genes:  
V $\kappa$ 1, V $\kappa$ 2, V $\kappa$ 3, V $\kappa$ 4, V $\lambda$ 1, V $\lambda$ 2, V $\lambda$ 3, VH1A, VH1B, VH2, VH3, VH4, VH5, VH6, C $\kappa$ , C $\lambda$ , CH1 or any combination of said HuCAL consensus genes.
23. The method according to any one of claims 20 to 22, wherein said derivative of said immunoglobulin family or said combination is an Fv, disulphide-linked Fv, single-chain Fv (scFv), or Fab fragment.

24. The method according to claims 22 to 23, wherein said derivative is an scFv fragment comprising the combination of HuCAL VH3 and HuCAL V $\lambda$ 2 consensus genes that comprises a random sub-sequence encoding the heavy chain CDR3 sub-element.
25. The method according to any one of claims 1 to 24, wherein at least part of said (poly)peptide sequences or (poly)peptides is connected to a sequence encoding at least one additional moiety or to at least one additional moiety, respectively.
26. The method according to claim 25, wherein said connection is formed via a contiguous nucleic acid sequence or amino acid sequence, respectively.
27. The method according to claims 25 to 26, wherein said additional moiety is a toxin, a cytokine, a reporter enzyme, a moiety being capable of binding a metal ion, a peptide, a tag suitable for detection and/or purification, or a homo- or hetero-association domain.
28. The method according to any one of claims 10 to 27, wherein the expression of said nucleic acid sequences results in the generation of a repertoire of biological activities and/or specificities, preferably in the generation of a repertoire based on a universal framework.
29. A nucleic acid sequence obtainable by the method according to any of claims 1 to 28.
30. A collection of nucleic acid sequences obtainable by the method according to any of claims 1 to 28.
31. A recombinant vector obtainable by the method according to any of claims 5 to 28.
32. A collection of recombinant vectors obtainable by the method according to any of claims 5 to 30.
33. A host cell transformed with the recombinant vector according to claim 31.



34. A collection of host cells transformed with the collection of recombinant vectors according to claim 32.
35. A method of producing a (poly)peptide or a collection of (poly)peptides as defined in any of claims 1 to 28 comprising culturing the host cell according to claim 33 or the collection of host cells according to claim 34 under suitable conditions and isolating said (poly)peptide or said collection of (poly)peptides.
36. A (poly)peptide devisable by the method according to any one of claims 1 to 3, encoded by the nucleic acid sequence according to claim 29 or obtainable by the method according to any one of claims 4 to 28 or 35.
37. A collection of (poly)peptides devisable by the method according to any one of claims 1 to 3, encoded by the collection of nucleic acid sequences according to claim 30 or obtainable by the method according to any one of claims 4 to 28 or 35.
38. A vector suitable for use in the method according to any of claims 5 to 28 and 35 characterized in that said vector is essentially devoid of any cleavage site as defined in claim 1(e) and 2.
39. The vector according to claim 38 which is an expression vector.
40. A kit comprising at least one of:
- (a) a nucleic acid sequence according to claim 29;
  - (b) a collection of nucleic acid sequences according to claim 30;
  - (c) a recombinant vector according to claim 31;
  - (d) a collection of recombinant vectors according to claim 32;
  - (e) a (poly)peptide according to claim 36;
  - (f) a collection of (poly)peptides according to claim 37;
  - (g) a vector according to claim 38 or 39; and optionally,
  - (h) a suitable host cell for carrying out the method according to claim 35.
41. A method of designing two or more genes encoding a collection of two or more proteins, comprising the steps of:

- (a) either
  - (aa) identifying two or more homologous gene sequences, or
  - (ab) analyzing at least three homologous genes, and deducing two or more consensus gene sequences therefrom,
- (b) optionally, modifying codons in said consensus gene sequences to remove unfavourable interactions between amino acids in the resulting proteins,
- (c) identifying sub-sequences which encode structural sub-elements in said consensus gene sequences
- (d) modifying one or more bases in regions adjacent to or between the ends of said sub-sequences to define one or more cleavage sites, each of which:
  - (da) are unique within each consensus gene sequence,
  - (db) do not form compatible sites with respect to any single sub-sequence,
  - (dc) are common to all homologous sub-sequences.

42. A method of preparing two or more genes encoding a collection of two or more proteins, comprising the steps of :

- (a) designing said genes according to claim 41, and
- (b) synthesizing said genes.

43. A collection of genes prepared according to the method of claim 42.

44. A collection of two or more genes derived from gene sequences which:

- (a) are either homologous, or represent consensus gene sequences derived from at least three homologous genes, and

- (b) carry cleavage sites, each of which:
  - (ba) lie at or adjacent to the ends of genetic sub-sequences which encode structural sub-elements,
  - (bb) are unique within each gene sequence,
  - (bc) do not form compatible sites with respect to any single sub-sequence, and
  - (bd) are common to all homologous sub-sequences.
- 45. The collection of genes according to either of claims 43 or 44 in which each of said gene sequences has a nucleotide composition characteristic of a particular species.
- 46. The collection of genes according to claim 45 in which said species is human.
- 47. The collection of genes according to any of claims 43 to 46 in which one or more of said gene sequences encodes at least part of a member of the immunoglobulin superfamily, preferably of the immunoglobulin family.
- 48. The collection of genes according to claim 47 in which said structural sub-elements correspond to any combination of framework regions 1, 2, 3, and 4, and/or CDR regions 1, 2, and 3 of antibody heavy chains.
- 49. The collection of genes according to claim 47 in which said structural sub-elements correspond to any combination of framework regions 1, 2, 3, and 4, and/or CDR regions 1, 2, and 3 of antibody light chains.
- 50. A collection of vectors comprising a collection of gene sequences according to any of claims 43 to 49.

51. The collection of vectors according to claim 50 comprising the additional feature that the vector does not comprise any cleavage site that is contained in the collection of genes according to any of claims 43 to 49.
52. A method for identifying one or more genes encoding one or more proteins having a desirable property, comprising the steps of:
- (a) expressing from the collection of vectors according to either of claims 50 or 51 a collection of proteins.
  - (b) screening said collection to isolate one or more proteins having a desired property,
  - (c) identifying the genes encoding the proteins isolated in step (b),
  - (d) optionally, excising from the genes encoding the proteins isolated in step (b) one or more genetic sub-sequences encoding structural sub-elements, and replacing said sub-sequence(s) by one or more second sub-sequences encoding structural sub-elements, to generate new vectors according to either of claims 50 or 51,
  - (e) optionally, repeating steps (a) to (c).
53. A method for identifying one or more genes encoding one or more antibody fragments which binds to a target, comprising the steps of:
- (a) expressing from the collection of vectors according to either of claims 50 or 51 a collection of proteins,
  - (b) screening said collection to isolate one or more antibody fragments which bind to said target,
  - (c) identifying the genes encoding the proteins isolated in step (b),
  - (d) optionally, excising from the genes encoding the antibody fragments isolated in step (b) one or more genetic sub-sequences encoding structural sub-elements, and replacing said sub-sequence(s) by one or

more second sub-sequences encoding structural sub-sequences new vectors according to either of claims 50 or 51,

(e) optionally, repeating steps (a) to (c).

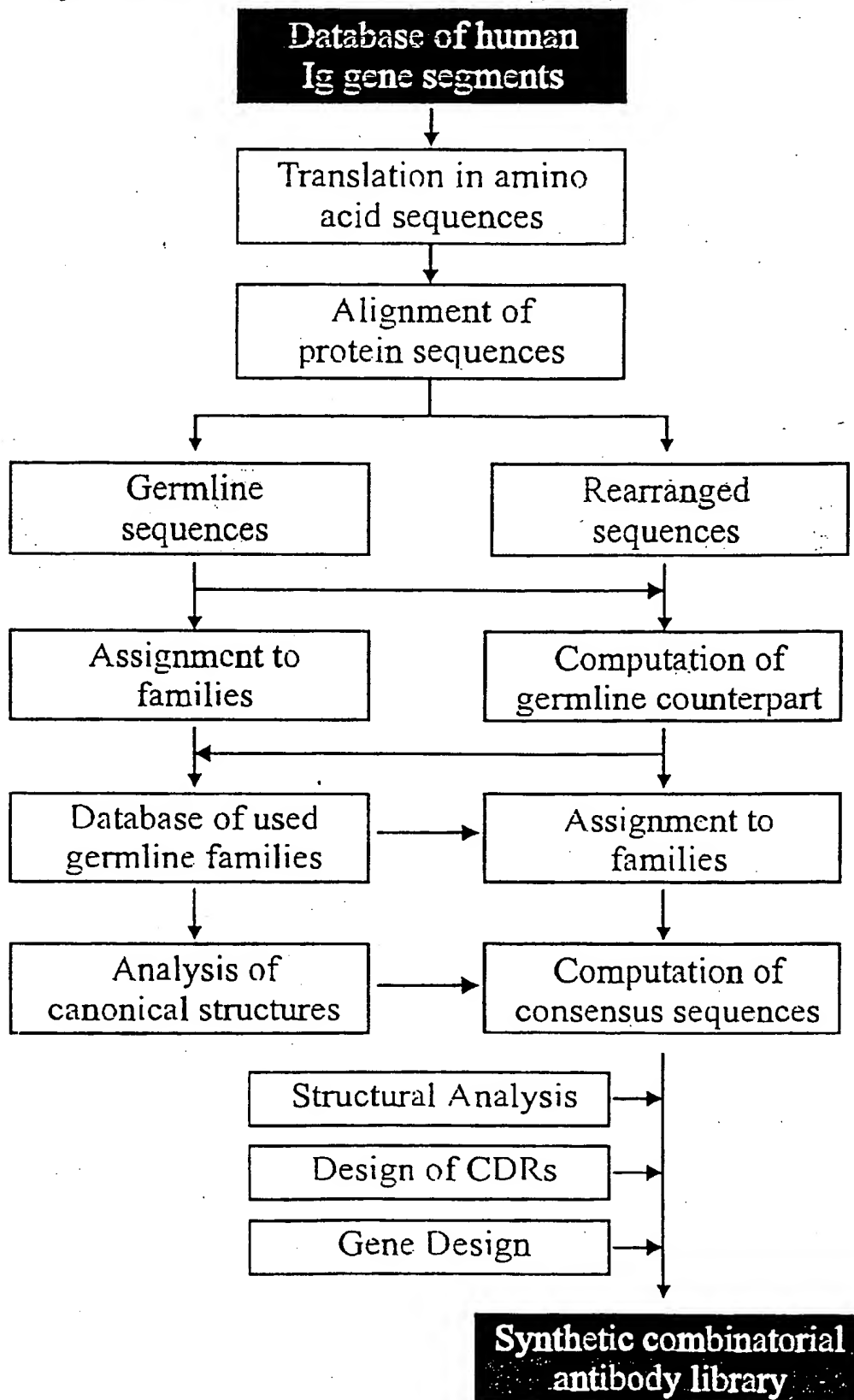
54. A kit comprising two or more genes derived from gene sequences which:

- (a) are either homologous, or represent consensus gene sequences derived from at least three homologous genes, and
- (b) carry cleavage sites, each of which:
  - (ba) lie at or adjacent to the ends of genetic sub-sequences which encode structural sub-elements,
  - (bb) are unique within each gene sequence,
  - (bc) do not form compatible sites with respect to any single sub-sequence, and
  - (bd) are common to all homologous sub-sequences.

55. A kit comprising two or more genetic sub-sequences which encode structural sub-elements, which can be assembled to form genes, and which carry cleavage sites, each of which:

- (a) lie at or adjacent to the ends of said genetic sub-sequences,
- (b) do not form compatible sites with respect to any single sub-sequence, and
- (d) are common to all homologous sub-sequences.

Figure 1: construction of a synthetic human antibody library based on consensus sequences



framework 1										CDRI																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	A	B	C
Vk1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	-	-	-
Vk2	D	I	V	M	T	Q	S	P	L	S	L	P	V	T	P	G	E	P	A	S	I	S	C	R	S	S	Q	S	L	L
Vk3	D	I	V	L	T	Q	S	P	A	T	L	S	L	S	P	G	E	R	A	T	L	S	C	R	A	S	Q	S	-	-
Vk4	D	I	V	M	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T	I	N	C	R	S	S	Q	S	V	L

CDRI			framework 2																			CDRII								
D	F	F	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
Vκ1	-	-	G	I	S	S	Y	L	A	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	A	A	S	S	L	
Vκ2	H	S	-	N	G	Y	N	Y	L	D	W	Y	L	Q	K	P	G	Q	S	P	Q	L	L	I	Y	L	G	S	N	R
Vκ3	-	-	-	V	S	S	Y	L	A	W	Y	Q	Q	K	P	G	Q	A	P	R	L	L	I	Y	G	A	S	S	R	
Vκ4	Y	S	S	N	N	K	N	Y	L	A	W	Y	Q	Q	K	P	G	Q	P	P	K	L	L	I	Y	W	A	S	T	R

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framework 3			CDRIII			framework 4																			
85	T	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
86	Y	Y	Y	Y	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
87	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
88	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
89	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
90	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
91	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
92	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
93	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
94	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
95	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
96	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
97	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
98	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
99	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
100	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
101	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
102	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
103	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
104	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
105	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
106	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
107	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
108	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T
109	Y	Y	Y	C	Q	Q	H	Y	T	T	P	P	T	F	G	Q	G	T	K	V	E	I	K	R	T



Figure 2B: VL lambda consensus sequences

framework 1														CDRI														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
VA1	Q	S	V	L	T	Q	P	P	S	-	V	S	G	A	P	G	Q	R	V	T	I	S	C	S	G	S	S	I
VA2	Q	S	A	L	T	Q	P	A	S	-	V	S	G	S	P	G	Q	S	I	T	I	S	C	T	G	T	S	S
VA3	S	Y	E	L	T	Q	P	P	S	-	V	S	V	A	P	G	Q	T	A	R	I	S	C	S	G	D	A	-

29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	framework 2														CDRI II													
VA1	G	S	N	-	Y	V	S	W	Y	Q	Q	L	P	G	T	A	P	K	L	L	I	Y	D	N	N	Q	R	P																				
VA2	G	G	Y	N	Y	V	S	W	Y	Q	Q	H	P	G	K	A	P	K	L	M	I	Y	D	V	S	N	R	P																				
VA3	G	D	K	-	Y	A	S	W	Y	Q	Q	K	P	G	Q	A	P	V	L	V	I	Y	D	D	S	D	R	P																				

Figure 2B: VL lambda consensus sequences

framework 3	
58	V
59	P
60	D
61	R
62	F
63	S
64	G
65	S
66	K
67	S
68	G
69	T
70	S
71	A
72	S
73	L
74	A
75	I
76	T
77	G
78	L
79	Q
80	S
81	E
82	D
83	E
84	A
85	D
86	Y
87	Y
VA1	
VA2	V
VA3	I
framework 4	
88	C
89	Q
90	Q
91	H
92	Y
93	T
94	T
95	P
96	P
97	V
98	F
99	G
100	G
101	G
102	T
103	K
104	L
105	T
106	V
107	L
VA1	
VA2	C
VA3	C

framework 1																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
VH1A	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	S	V	K	V	S	C	K	A	S	G	G	T	F	S		
VH1B	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	T	F	T	
VH2	Q	V	Q	L	K	E	S	G	P	A	L	V	K	P	T	Q	T	L	T	L	T	C	T	F	S	G	F	S	L	S	
VH3	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	S	
VH4	Q	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	E	T	L	S	L	T	C	T	V	S	G	G	S	I	S	
VH5	E	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	E	S	L	K	I	S	C	K	G	S	G	Y	S	F	T	
VH6	Q	V	Q	L	Q	Q	S	G	P	G	L	V	K	P	S	Q	T	L	S	L	T	C	A	I	S	G	D	S	V	S	

CDRI			framework 2										CDR II																			
31	A	B	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55	56	57	
VH1A	S	-	Y	A	I	S	W	V	R	Q	A	P	G	Q	G	L	E	W	M	G	G	I	I	P	-	-	I	F	G	T	A	
VH1B	S	-	Y	Y	M	H	W	V	R	Q	A	P	G	Q	G	L	E	W	M	G	W	I	N	P	-	-	N	S	G	G	T	
VH2	T	S	G	V	G	V	I	R	Q	P	P	G	K	A	L	E	W	L	A	L	I	D	-	-	-	-	W	D	D	D	K	
VH3	S	-	Y	A	M	S	W	V	R	Q	A	P	G	K	G	L	E	W	V	S	A	I	S	G	-	-	S	G	G	S	T	
VH4	S	-	Y	Y	W	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G	Y	I	Y	-	-	-	Y	S	G	S	T	
VH5	S	-	Y	W	I	G	W	V	R	Q	M	P	G	K	G	L	E	W	M	G	I	I	Y	P	-	-	G	D	S	D	T	
VH6	S	N	S	A	A	W	N	W	I	R	Q	S	P	G	R	G	L	E	W	L	G	R	T	Y	Y	R	-	S	K	W	Y	N

Figure 2C: V heavy chain consensus sequences

CDRII										framework 3																					
58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85				
VH1A	N	Y	A	Q	K	F	Q	G	R	V	T	I	T	A	D	E	S	T	S	T	A	Y	M	E	L	S	S	L	R	S	E
VH1B	N	Y	A	Q	K	F	Q	G	R	V	T	M	T	R	D	T	S	I	S	T	A	Y	M	E	L	S	S	L	R	S	E
VH2	Y	Y	S	T	S	L	K	T	R	L	T	I	S	K	D	T	S	K	N	Q	V	V	L	T	M	T	N	M	D	P	V
VH3	Y	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E
VH4	N	Y	N	P	S	L	K	S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A
VH5	R	Y	S	P	S	F	Q	G	Q	V	T	I	S	A	D	K	S	I	S	T	A	Y	L	Q	W	S	S	L	K	A	S
VH6	D	Y	A	V	S	V	K	S	R	I	T	I	N	P	D	T	S	K	N	Q	F	S	L	Q	L	N	S	V	T	P	E

framework 3										CDRIII										framework 4											
86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	A	B	C	101	102	103	104	105	106	107	108	109	110	111	112	113	
VH1A	D	T	A	V	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH1B	D	T	A	V	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH2	D	T	A	T	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH3	D	T	A	V	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH4	D	T	A	V	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH5	D	T	A	M	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S
VH6	D	T	A	V	Y	Y	C	A	R	W	G	G	D	G	F	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S	S

Figure 3A: V kappa 1 (Vk1) gene sequence

```

.D I Q M T Q S P S S L S A S V G D
EcoRV                               BanII
~~~~~                               ~~~~~
GATATCCAGA TGACCCAGAG CCCGTCTAGC CTGAGCGCGA GCGTGGGTGA
CTATAGGTCT ACTGGGTCTC GGCAGATCG GACTCGCGCT CGCACCCACT

R V T I T C R A S Q G I S S Y L
PstI
~~~~~
TCGTGTGACC ATTACCTGCA GAGCGAGCCA GGCATTAGC AGCTATCTGG
AGCACACTGG TAATGGACGT CTCGCTCGGT CCCGTAATCG TCGATAGACC

A W Y Q Q K P G K A P K L L I Y A
KpnI                               SexAI
~~~~~                               ~~~~~
CGTGGTACCA GCAGAAACCA GGTAAGCAC CGAAACTATT AATTATGCA
GCACCATGGT CGTCTTTGGT CCATTTCGTG GCTTTGATAA TTAAATACGT

A S S L Q S G V P S R F S G S
SandI                               BamHI
~~~~~                               ~~~~~
GCCAGCAGCT TGCAAAGCGG GGTCCCCTCC CGTTTAGCG GCTCTGGATC

```

Figure 3A: V kappa 1 (Vk1) gene sequence (continued)

CGGTCGTCGA ACGTTTCGCC CCAGGGCAGG GCAAATCGC CGAGACCTAG

G T D F T L T I S S L Q P E D F

Eco57I

~~~~~

BamHI

BbsI

~~~~~

CGGCACTGAT TTACCCTGA CCATTAGCAG CCTGCAACCT GAAGACTTTG  
GCCGTGACTA AAATGGGACT GGTAATCGTC GGACGTTGGA CTTCTGAAAC

A T Y Y C Q Q H Y T T P P T F G Q

MSCI

~~~~~

CGACCTATTA TTGCCAGCAG CATTATACCA CCCC GCCGAC CTTTGGCCAG  
GCTGGATAAT AACGGTCGTC GTAATATGTT GGGGCGGCTG GAAACCGGTC

G T K V E I K R T

BsiWI

~~~~~

GGTACGAAAG TTGAAATTAA ACGTACG  
CCATGCTTTC AACTTTAATT TGCATGC

Figure 3B: V kappa 2 (Vκ2) gene sequence

```

D I V M T Q S P L S L P V T P G E
EcoRV      BanII
~~~~~
GATATCGTGA TGACCCAGAG CCCACTGAGC CTGCCAGTGA CTCCGGGCGA
CTATAGCACT ACTGGGTCTC GGTGACTCG GACGGTCACT GAGGCCCGCT

P A S I S C R S S Q S L L H S N
PstI
~~~~~
GCCTGCGAGC ATTAGCTGCA GAAGCAGCCA AAGCCTGCTG CATAGCAACG
CGGACGCTCG TAATCGACGT CTTCGTCGGT TTCGGACGAC GTATCGTTGC

G Y N Y L D W Y L Q K P G Q S P Q
KpnI      SexAI
~~~~~
GCTATAACTA TCTGGATTGG TACCTTCAAA AACCAGGTCA AAGCCCGCAG
CGATATTGAT AGACCTAACC ATGGAAGTTT TTGGTCCAGT TTCGGGCGTC

L L I Y L G S N R A S G V P D R F
AseI      SmaI
~~~~~
CTATTAAATT ATCTGGGCAG CAACCGTGCC AGTGGGGTCC CGGATCGTTT
GATAATTAAA TAGACCCGTC GTTGGCACGG TCACCCCGAG GCCTAGCCAA

```

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Figure 3B: V kappa 2 (Vk2) gene sequence (continued)

S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V
BamHI															
~~~~~															
TAGCGGCTCT	GGATCCGGCA	CCGATTTTAC	CCTGAAAATT	AGCCGTGTGG											
ATCGCCGAGA	CCTAGGCCGT	GGCTAAAATG	GGACTTTTAA	TCGGCACACC											
E	A	E	D	V	G	V	Y	Y	C	Q	Q	H	Y	T	T
Eco57I															
~~~~~															
BbsI															
~~~~~															
AAGCTGAAGA	CGTGGGCGTG	TATTATTGCC	AGCAGCATTA	TACCACCCCG											
TTCGACTTCT	GCACCCGCAC	ATAATAACGG	TCGTCGTAAT	ATGTTGGGC											
P	T	F	G	Q	G	T	K	V	E	I	K	R	T		
MscI															
~~~~~															
CCGACCTTTG	GCCAGGGTAC	GAAAGTTGAA	ATTAAACGTA	CG											
GGCTGGAAAC	CGGTCCCATG	CTTCAACTT	TAATTGTCAT	GC											
BsiWI															
~~~~~															



Figure 3C: V kappa 3 (Vk3) gene sequence

```

D I V L T Q S P A T L S L S P G E
EcoRV                               BanII
~~~~~                               ~~~~~
GATATCGTGC TGACCCAGAG CCCGGCGACC CTGAGCCTGT CTCCGGGCGGA
CTATAGCAGC ACTGGGTCTC GGGCCGCTGG GACTCGGACA GAGGCCCGCT

R A T L S C R A S Q S V S S Y
                               PstI
                               ~~~~~
ACGTGCGACC CTGAGCTGCA GAGCGAGCCA GAGCGTGAGC AGCAGCTATC
TGCACGCTGG GACTCGACGT CTCGCTCGGT CTCGCACTCG TCGTCGATAG

L A W Y Q Q K P G Q A P R L L I Y
KpnI                               SexAI                               AseI
~~~~~                               ~~~~~                               ~~~~~
TGGCGTGGTA CCAGCAGAAA CCAGGTCAAG CACCGCGTCT ATTAATTAT
ACCGCACCAT GGTCTCTTT GTCCAGTTC GTGGCGCAGA TAATTAATA

G A S S R A T G V P A R F S G S G
                               SandI                               BamHI
                               ~~~~~                               ~~~
GGCGCGAGCA GCCGTGCAAC TGGGTCCCG GCGCGTTTA GCGGCTCTGG

```

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Figure 3C: V kappa 3 (Vk3) gene sequence (continued)

CCGCGGCTCGT CGGCACGTTG ACCCCAGGGC CGCGCAAAAT CGCCGAGACC

S G T D F T L T I S S L E P E D  
Eco57I  
~~~~~

BbsI

BamHI

~~~~~  
ATCCGGCAG GATTTACCC TGACCATTAG CAGCCTGGAA CCTGAAGACT  
TAGGCCGTGC CTAAATGGG ACTGGTAATC GTCGGACCTT GGACTTCTGA

F A V Y Y C Q Q H Y T T P P T F G  
MscI  
~~~~~

TTGCGGTGTA TTATTGCCAG CAGCATTATA CCACCCCGCC GACCTTTGGC  
AACGCCACAT AATAACGGTC GTCGTAATAT GGTGGGGCGG CTGGAAACCG

Q G T K V E I K R T  
MscI BsiWI  
~~~~~

CAGGTACGA AAGTTGAAAT TAAACGTACG  
GTCCCATGCT TTCAACTTA ATTTGCATGC

Figure 3D: V kappa 4 (Vx4) gene sequence

```

D I V M T Q S P D S L A V S L G E
EcoRV                               BanII
~~~~~
GATATCGTGA TGACCCAGAG CCCGATAGC CTGGCGGTGA GCCTGGGCGA
CTATAGCACT ACTGGGTCTC GGCCTATCG GACCGCCACT CGGACCCGCT

R A T I N C R S S Q S V L Y S S
PstI
~~~~~
ACGTGCGACC ATTAAGTGA GAAGAGCCA GAGCGTGCTG TATAGCAGCA
TGCACGCTGG TAATTGACGT CTCGTCGGT CTCGCACGAC ATATCGTCGT

N N K N Y L A W Y Q Q K P G Q P P
KpnI                               SexAI
~~~~~
ACAACAAAA CTATCTGGCG TGGTACCAGC AGAAACCAGG TCAGCCGCCG
TGTTGTTTTT GATAGACCGC ACCATGGTCG TCTTTGGTCC AGTCGGCGGC

K L L I Y W A S T R E S G V P D R
AseI                               SmaI
~~~~~
AAACTATTAA TTTATTGGG ATCCACCCGT GAAAGCGGG TCCCGGATCG
TTTGATAATT AAATAACCCG TAGTGGGCA CTTTCGCCCC AGGCCCTAGC

```

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Figure 3D: V kappa 4 (Vk4) gene sequence (continued)

```

F S G S G S G T D F T L T I S S
      BamHI
TTTTAGCGGC TCTGGATCCG GCACTGATTT TACCCTGACC ATTTCGTCCC
AAATCGCCG AGACCTAGGC CGTGACTAAA ATGGGACTGG TAAAGCAGGG

L Q A E D V A V Y Y C Q Q H Y T T
      Eco57I
~~~~~
      BbsI
~~~~~
TGCAAGCTGA AGACGTGGCG GTGTATTATT GCCAGCAGCA TTATACCACC
ACGTTGCACT TCTGCACCGC CACATAATAA CGGTCGTCGT AATATGGTGG

P P T F G Q G T K V E I K R T
      MscI
~~~~~
      BsiWI
~~~~~
CCGCCGACCT TTGGCCAGGG TACGAAAGTT GAAATTAAAC GTACG
GGCGGCTGGA AACCGGTCCC ATGCTTTCAA CTTTAATTG CATGC

```

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re 4A: V lambda 1 (V<sub>λ1</sub>) gene sequence

CAGAGCGTGC TGACCCAGCC GCCTTCAGTG AGTGGCGCAC CAGGTCAGCG  
 GTCTCGCACG ACTGGGTCGG CGGAAGTCAC TCACCGCGTG GTCCAGTCCG  
 Eco57I  
 ~~~~~

V T I S C S G S S S N I G S N Y  
BSSSI

~~~~~  
TGTGACCATC TCGTGTAGCG GCAGCAGCAG CAACATTGGC AGCAACTATG  
ACACTGGTAG AGCACATCGC CGTCGTCGTC GTTGTAACCG TCGTTGATAC

V S W Y Q Q Q L P G T A P K L L I Y  
KpnI XmaI BbeI

~~~~~  
TGAGCTGGTA CCAGCAGTTG CCCGGACGG CGCCGAACT GCTGATTAT  
ACTCGACCAT GGTCTCAAC GGGCCCTGCC GCGGCTTGA CGACTAAATA

D N N Q R P S G V P D R F S G S K  
Bsu36I BamHI

2  
2  
2  
2  
2  
2

Figure 4A: V lambda 1 (Vλ1) gene sequence (continued)

```

GATAACAACC AGCGTCCCTC AGGCGTGCCG GATCGTTTAA GCGGATCCAA
CTATTGTTGG TCGCAGGGAG TCCGCACGGC CTAGCAAAAT CGCCTAGGTT

      S  G  T  S  A  S  L  A  I  T  G  L  Q  S  E  D
                                     BbsI
                                     ~~~~~
AAGCGGCACC AGCGCGAGCC TTGCGATTAC GGGCCTGCAA AGCGAAGACG
TTCGCCCGTGG TCGCGCTCGG AACGCTAATG CCCGGACGTT TCGCTTCTGC

      E  A  D  Y  Y  C  Q  Q  H  Y  T  T  P  P  V  F  G
AAGCGGATTA TTATTGCCAG CAGCATTATA CCACCCCGCC TGTGTTTGGC
TTCGCCCTAAT AATAACGGTC GTCGTAATAT GGTGGGCGG ACACAAACCG

      G  G  T  K  L  T  V  L  G
                HpaI      MscI
                ~~~~~
GGCGGCACGA AGTTAACCGT TCTTGGC
CCGCCGTGCT TCAATTGGCA AGAACCG

```

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| Q          | S | A | L          | T | Q | P          | A | S | V      | S          | G | S | P           | G | Q | S |
|------------|---|---|------------|---|---|------------|---|---|--------|------------|---|---|-------------|---|---|---|
|            |   |   |            |   |   |            |   |   |        |            |   |   | SexAI       |   |   |   |
|            |   |   |            |   |   |            |   |   |        |            |   |   | ~~~~~       |   |   |   |
| CAGAGCGCAC |   |   | TGACCCAGCC |   |   | AGCTTCAGTG |   |   |        | AGCGGCTCAC |   |   | CAGGTCAGAG  |   |   |   |
| GTCTCGCGTG |   |   | ACTGGGTCGG |   |   | TCGAAGTCAC |   |   |        | TCGCCGAGTG |   |   | GTCACAGTCTC |   |   |   |
|            |   |   |            |   |   |            |   |   | Eco57I |            |   |   |             |   |   |   |
|            |   |   |            |   |   |            |   |   | ~~~~~  |            |   |   |             |   |   |   |

|            |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
|------------|------------|------------|------------|---------|------------|---------|---------|------|---|---|---|---|---|---|---|
| I          | T          | I          | S          | C       | T          | G       | T       | S    | S | D | V | G | G | Y | N |
| BssSI      |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
| ~~~~~      |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
| CATTACCATC | TCGTGTA    | CCGG       | GTACTAG    | CAG     | CGATGTG    | GGC     | GGCTATA | AACT |   |   |   |   |   |   |   |
| GTAATGGTAG | AGCACATGCC | CATGATCGTC | GCTACAC    | CCCG    | CCGATAT    | TGA     |         |      |   |   |   |   |   |   |   |
| Y          | V          | S          | W          | Y       | Q          | Q       | H       | P    | G | K | A | P | K | L | M |
| KpnI       |            |            |            | XmaI    |            |         |         | BbeI |   |   |   |   |   |   |   |
| ~~~~~      |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
| ATGTGAGCTG | GTACCAGCAG | CATCCCCGGA | AGGC       | GGCCGAA | ACTGATG    | ATT     |         |      |   |   |   |   |   |   |   |
| TACACTCGAC | CATGGTCGTC | GTAGGGCCCT | TCCG       | CGGCTT  | TGACTACTAA |         |         |      |   |   |   |   |   |   |   |
| Y          | D          | V          | S          | N       | R          | P       | S       | G    | V | S | N | R | F | S | G |
| Bsui36I    |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
| ~~~~~      |            |            |            |         |            |         |         |      |   |   |   |   |   |   |   |
| TATGATGTGA | GCAACCGTCC | CTCAGGCGTG | AGCA       | AACCGTT | TTAGCGG    | ATC     |         |      |   |   |   |   |   |   |   |
| ATACTA     | CACT       | CGTTGGCAGG | GAGTCCGCAC | TCGT    | TGGCAA     | AATCGCC | CTAG    |      |   |   |   |   |   |   |   |

Figure 4B: V lambda 2 (Vλ2) gene sequence (continued)

```

      K  S  G  N  T  A  S  L  T  I  S  G  L  Q  A  E
      BamHI
      ~~~~~
      CAAAGCGGC AACACCGCGA GCCTGACCAT TAGCGGCCCTG CAAGCGGAAG
      GTTTTCGCCG TTGTGGCGCT CGGACTGGTA ATCGCCGGAC GTTCGCCCTTC

      D  E  A  D  Y  Y  C  Q  Q  H  Y  T  T  P  P  V  F
      BbsI
      ~~~~~
      ACGAAGCGGA TTATTATTGC CAGCAGCATT ATACCACCCC GCCTGTGTTT
      TGCTTCGCCCT AATAATAACG GTCGTCGTAA TATGGTGGG CGGACACAAA

      G  G  G  T  K  L  T  V  L  G
      HpaI      MscI
      ~~~~~
      GCGGCGGCA CGAAGTTAAC CGTTCTTGGC
      CCGCCGCCGT GCTTCAATTG GCAAGAACCG

```

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Figure 4C: V lambda 3 (Vλ3) gene sequence

|  |            |            |            |            |   |   |   |   |   |   |   |   |   |   |   |   |
|--|------------|------------|------------|------------|---|---|---|---|---|---|---|---|---|---|---|---|
| S  | Y          | E          | L          | T          | Q | P | P | S | V | S | V | A | P | G | Q | T |
| <div style="text-align: center;">SexAI</div> <div style="text-align: center;">~~~~~</div>  |            |            |            |            |   |   |   |   |   |   |   |   |   |   |   |   |
| AGCTATGAAC   | TGACCCAGCC | GCCTTCAGTG | AGCGTTGCAC | CAGGTCAGAC |   |   |   |   |   |   |   |   |   |   |   |   |
| TCGATACTTG   | ACTGGGTCGG | CGGAAGTCAC | TCGCAACGTG | GTCCAGTCTG |   |   |   |   |   |   |   |   |   |   |   |   |
| <div style="text-align: center;">Eco57I</div> <div style="text-align: center;">~~~~~</div>   |            |            |            |            |   |   |   |   |   |   |   |   |   |   |   |   |
| A  | R          | I          | S          | C          | S | G | D | A | L | G | D | K | Y | A | S |   |
| <div style="text-align: center;">BssSI</div> <div style="text-align: center;">~~~~~</div>  |            |            |            |            |   |   |   |   |   |   |   |   |   |   |   |   |
| CGCGCGTATC   | TCGTGTAGCG | GCGATGCGCT | GGCGGATAAA | TACGCGAGCT |   |   |   |   |   |   |   |   |   |   |   |   |
| GCGCGCATAG   | AGCACATCGC | CGCTACGCGA | CCCGCTATT  | ATGCGCTCGA |   |   |   |   |   |   |   |   |   |   |   |   |
| W  | Y          | Q          | Q          | K          | P | G | Q | A | P | V | L | V | I | Y | D | D |
| <div style="text-align: center;">KpnI</div> <div style="text-align: center;">~~~~~</div> <div style="text-align: center;">XmaI</div> <div style="text-align: center;">~~~~~</div> <div style="text-align: center;">BbeI</div> <div style="text-align: center;">~~~~~</div> |            |            |            |            |   |   |   |   |   |   |   |   |   |   |   |   |
| GGTACCAGCA   | GAAACCCGGG | CAGGCGCCAG | TTCTGGTGAT | TTATGATGAT |   |   |   |   |   |   |   |   |   |   |   |   |
| CCATGGTCGT   | CTTTGGGCCC | GTCCGCGGTC | AAGACCACTA | AATACTACTA |   |   |   |   |   |   |   |   |   |   |   |   |

Figure 4C: V lambda 3 (λ3) gene sequence (continued)

```

S D R P S G I P E R F S G S N S G
      Bsu36I      BamHI
      ~~~~~
TCTGACCGTC CCTCAGGCAT CCCGGAACGC TTTAGCCGGAT CCAACAGCGG
AGACTGGCAG GGAGTCCGTA GGCCTTGCG AAATCGCCTA GGTGTGCGCC

N T A T L T I S G T Q A E D E A
      BbsI
      ~~~~~
CAACACCGCG ACCCTGACCA TTAGCGGCAC TCAGGCGGAA GACGAAGCGG
GTTGTGGCGC TGGGACTGGT AATCGCCGTG AGTCCGCCTT CTGCTTCGCC

D Y Y C Q Q H Y T T P P V F G G G
ATTATTATTG CCAGCAGCAT TATACCACCC CGCCTGTGTT TGGCGGCGGC
TAATAATAAC GGTCGTCGTA ATATGTTGG GCGACACAA ACCGCCGCCG

T K L T V L G
      HpaI      MscI
      ~~~~~
ACGAAGTTAA CCGTTC TTGG C
TGCTTCAATT GCAAGAACC G

```

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Figure 5A: V heavy chain 1A (VH1A) gene sequence

```

Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  S  S
MfeI
~~~~~
CAGGTGCAAT TGGTTCAGTC TGGCGCGGAA GTGAAAAAAC CGGGCAGCAG
GTCCACGTTA ACCAAGTCAG ACCGCGCCTT CACTTTTGTG GCCCGTCGTC

V  K  V  S  C  K  A  S  G  G  T  F  S  S  Y  A
BspEI
~~~~~
CGTGAAAGTG AGCTGCAAAAG CCTCCGGAGG CACTTTTAGC AGCTATGCCA
GCACTTTCAC TCGACGTTTC GGAGGCCTCC GTGAAAATCG TCGATACGCT

I  S  W  V  R  Q  A  P  G  Q  G  L  E  W  M  G  G
BstXI
~~~~~
XhoI
~~~~~
TTAGCTGGGT GCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCGGC
AATCGACCCA CGCGGTTCCG GGACCCGCTCC CAGAGCTCAC CTACCCGCGC

I  I  P  I  F  G  T  A  N  Y  A  Q  K  F  Q  G  R
ATTATTCCGA TTTTGGCAC GGCGAACTAC GCGCAGAAGT TTCAGGGCCG
TAATAAGGCT AAAAACCGTG CCGCTTGATG CGCGTCTTCA AAGTCCCGGC

V  T  I  T  A  D  E  S  T  S  T  A  Y  M  E  L
BstEII

```

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Figure 5A: V heavy chain 1A (VH1A) gene sequence (continued)

```

~~~~~
GGTGACCATT ACCGCGGATG AAAGCACCAG CACCGCGTAT ATGGAACCTGA
C CACTGGTAA TGGCGCCTAC TTTCGTGGTC GTGGCGCATA TACCTTGACT

S S L R S E D T A V Y Y C A R W G
      EagI      BssHII
      ~~~~~
GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTTGGGGC
CGTCGGACGC ATCGCTTCTA TGCCGGCACA TAATAACGCG CGCAACCCCG

G D G F Y A M D Y W G Q G T L V T
      StyI
      ~~~~~
GGCGATGGCT TTTATGCCAT GGATTATTGG GGCCAAGGCA CCCTGGTGAC
CCGCTACCGA AAATACGCTA CCTAATAACC CCGGTTCCGT GGGACCACTG

V S S
      BlnI
      ~~~~~
GGTTAGCTCA G
CCAATCGAGT C

```

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Figure 5B: V heavy chain 1B (VH1B) gene sequence

```

Q V Q L V Q S G A E V K K P G A S
MfeI
-----
CAGGTGCAAT TGGTTCAGAG CGGCGCGGAA GTGAAAAAAC CGGGCGCGGAG
GCCACGTTA ACCAAGTCTC GCCGCGCCTT CACTTTTGTG GCCCGCGCTC

V K V S C K A S G Y T F T S Y Y
BspEI
-----
CGTGAAAGTG AGCTGCAAAG CCTCCGGATA TACCTTTACC AGCTATTATA
GCACTTTCAC TCGACGTTTC GGAGGCCCTAT ATGGAAATGG TCGATAATAT

M H W V R Q A P G Q G L E W M G W
BstXI
-----
XhoI
-----
TGCAC TGGGT CCGCCAAGCC CCTGGGCAGG GTCTCGAGTG GATGGGCTGG
ACGTGACCCA GCGGTTTCGG GGACCCGTCC CAGAGCTCAC CTACCCGACC

I N P N S G G T N Y A Q K F Q G R
ATAAACC CGA ATAGGGCGG CACGAAC TAC GCGCAGAAGT TTCAGGGCCG
TAATTGGGCT TATCGCCGCC GTGCTTGATG CGCGTCTTCA AAGTCCCGGC

```

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Figure 5B: V heavy chain 1B (VH1B) gene sequence (continued)

```

V   T   M   T   R   D   T   S   I   S   T   A   Y   M   E   L
BstEII
~~~~~
GGTGACCATG ACCCGTGATA CCAGCATTAG CACCGCGTAT ATGGAACCTGA
CCACTGGTAC TGGGCACTAT GGTGTAATC GTGGCGCATA TACCTTGACT

S   S   L   R   S   E   D   T   A   V   Y   Y   C   A   R   W   G
      EagI
      ~~~~~
      BssHII
      ~~~~~
GCAGCCTGCG TAGCGAAGAT ACGGCCGTGT ATTATTGCGC GCGTTGGGGC
CGTCGGACGC ATCGCTTCTA TGCCGGCACA TAATAACGCG CGCAACCCCG

G   D   G   F   Y   A   M   D   Y   W   G   Q   G   T   L   V   T
      StyI
      ~~~~~
GGCGATGGCT TTTATGCGAT GGATTATTGG GGCCAAGGCA CCCTGGTGAC
CCGCTACCGA AAATACGCTA CCTAATAACC CCGGTTCCGT GGGACCACTG

V   S   S
      BlnI
      ~~~~~
GGTTAGCTCA G
CCAATCGAGT C

```

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Figure 5C: V heavy chain 2 (VH2) gene sequence

```

Q  V  Q  L  K  E  S  G  P  A  L  V  K  P  T  Q  T
      MfeI
~~~~~
CAGGTGCAAT TGAAGAAAG CGGCCCCGCC CTGGTGAAAC CGACCCAAAC
GTCCACGTTA ACTTCTTTC GCCGGGCCGG GACCACTTG GCTGGGTTTG

L  T  L  T  C  T  F  S  G  F  S  L  S  T  S  G
      BspEI
~~~~~
CCTGACCCCTG ACCTGTACCT TTTCGGGATT TAGCCTGTCC ACGTCTGGCG
GGA CTGGGAC TGGACATGGA AAAGGCCTAA ATCGGACAGG TGCAGACCGC

V  G  V  G  W  I  R  Q  P  P  G  K  A  L  E  W  L
      BstXI           XhoI
~~~~~
TTGGCGTGGG CTGGATTCCG CAGCCGCCCTG GGAAGCCCT CGAGTGGCTG
AACCGCACCC GACCTAAGCG GTCGGCGGAC CCTTTCGGGA GCTCACCCGAC

A  L  I  D  W  D  D  D  K  Y  Y  S  T  S  L  K  T
      MluI
~~~~~
GCTCTGATTG ATTGGGATGA TGATAAGTAT TATAGACCA GCCTGAAAC
CGAGACTAAC TAACCCTACT ACTATTCATA ATATCGTGGT CGGACTTTTG

```

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Figure 5C: V heavy chain 2 (VH2) gene sequence (continued)

```

R L T I S K D T S K N Q V V L T
MluI
-----
GCGTCTGACC ATTAGCAAAG ATACTTCGAA AAATCAGGTG GTGCTGACTA
CGCAGACTGG TAATCGTTTC TATGAAGCTT TTAGTCCAC CACGACTGAT

M T N M D P V D T A T Y Y C A R W
                               BssHII
                               -----
TGACCAACAT GGACCCGGTG GATACGGCCA CCTATTATTG CGCGCGTTGG
ACTGGTTGTA CCTGGGCCAC CTATGCCGGT GGATAATAAC GCGGCAACC

G G D G F Y A M D Y W G Q G T L V
                               StyI
                               -----
GGCGGCGATG GCTTTTATGC GATGGATTAT TGGGGCCAAG GCACCCCTGGT
CCGCCGCTAC CGAAATACG CTACCTAATA ACCCCGGTTC CGTGGGACCA

T V S S
    BlnI
    -----
GACGGTTAGC TCAG
CTGCCAATCG AGTC

```

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Figure 5D: V heavy chain 3 (VH3) gene sequence

```

E V Q L V E S G G G L V Q P G G S
MfeI
-----
GAAGTGCAAT TGGTGGAAG CGGCGGCGGC CTGGTGCAAC CGGCGGCGCAG
CTTCACGTTA ACCACCTTTC GCCGCCGCCG GACCACGTTG GCCCGCCGTC

L R L S C A A S G F T F S S Y A
BspEI
-----
CCTGCGTCTG AGTGCGCGG CCTCCGGATT TACCTTTAGC AGCTATGCCA
GGACGCAGAC TCGACGCGC GGAGGCCTAA ATGGAATCG TCGATACGCT

M S W V R Q A P G K G L E W V S A
BstXI
-----
XhoI
-----
TGAGCTGGGT GCGCCAAGCC CCTGGGAAGG GTCTCGAGTG GTGAGCGCGC
ACTCGACCCA CGCGGTTCCG GGACCCCTCC CAGAGCTCAC CCACTCGCGC

I S G S G G S T Y Y A D S V K G R
ATTAGCGGTA GCGCGGCGCAG CACCTATTAT GCGGATAGCG TGAAAGGCCG
TAATCGCCAT CGCCGCCGTC GTGGATAATA CGCCTATCGC ACTTCCGGC

```

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Figure 5D: V heavy chain 3 (VH3) gene sequence (continued)

```

F T I S R D N S K N T L Y L Q M
      PmlI NspV
TTTTACCAT TCACGTGATA ATCGAAAA CACCCTGTAT CTGCAAATGA
AAATGGTAA AGTGCACTAT TAAGCTTTT GTGGGACATA GACGTTTACT
N S L R A E D T A V Y Y C A R W G
      EagI BssHII
ACAGCCTGCG TCGGAAGAT ACGGCCGTGT ATTATTGCGC GCGTTGGGC
TGTCGGACGC ACGCCTTCTA TGCCGGCACA TAATAACGCG CGCAACCCCG
G D G F Y A M D Y W G Q G T L V T
      StyI
GGCGATGGCT TTTATGCGAT GGATTATTGG GGCCAAGGCA CCCTGGTGAC
CCGCTACCGA AAATACGCTA CCTAATAACC CCGGTTCCGT GGGACCACTG
V S S
      BlnI
GGTAGCTCA G
CCAATCGAGT C

```

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Figure 5E: V heavy chain 4 (VH4) gene sequence

```

Q  V  Q  L  Q  E  S  G  P  G  L  V  K  P  S  E  T
      MfeI
      ~~~~~
CAGGTGCAAT TGCAAGAAAG TGGTCCGGGC CTGGTGA AAC CGAGCGAAAC
GTCCACGTTA ACGTCTTTC ACCAGGCCCG GACCACTTG GCTCGCTTG

L  S  L  T  C  T  V  S  G  G  S  I  S  S  Y  Y
      BspEI
      ~~~~~
CCTGAGCCTG ACCTGCACCG TTCCGGAGG CAGCATTAGC AGCTATTATT
GGACTCGGAC TGGACGTGGC AAAGGCCTCC GTCGTAATCG TCGATAATAA

W  S  W  I  R  Q  P  P  G  K  G  L  E  W  I  G  Y
      BstXI
      ~~~~~
      XhoI
      ~~~~~
GGAGCTGGAT TCGCCAGCCG CCTGGGAAGG GTCTCGAGTG GATTGGCTAT
CCTCGACCCTA AGCGGTCGGC GGACCCTTCC CAGAGCTCAC CTAACCGATA

I  Y  Y  S  G  S  T  N  Y  N  P  S  L  K  S  R  V
      BstEII
      ~~~~~
ATTTATTATA GCGGCAGCAC CAACTATAAT CCGAGCCCTGA AAAGCCGGGT
TAAATAATAT CGCCGTCGTG GTTGATATTA GGCTCGGACT TTTCGGCCCA

```

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Figure 5E: V heavy chain 4 (VH4) gene sequence (continued)

```

T I S V D T S K N Q F S L K L S
BstEII
~~~~~
NSpV
~~~~~
GACCATTAGC GTTGATACTT CGAAAAACCA GTTAGCCTG AAAC TGAGCA
CTGGTAATCG CAACTATGAA GCTTTTGGT CAAATCGGAC TTTGACTCGT

S V T A A D T A V Y Y C A R W G G
      Egi
~~~~~
BSSHII
~~~~~
GCGTGACGGC GCGGATACG GCCGTGTATT ATTGCGCGCG TTGGGGCGGC
CGCACTGCCG CCGCCTATGC CGGCACATAA TAACGCGCGC AACCCCGCCG

D G F Y A M D Y W G Q G T L V T V
      StyI
~~~~~
GATGGCTTTT ATGCGATGGA TTATTGGGGC CAAGGCACCC TGGTGACGGT
CTACCGAAAA TACGCTACCT AATAACCCCG GTTCCGTGGG ACCACTGCCA

S S
BlpI
~~~~~
TAGCTCAG
ATCGAGTC

```

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Figure 5F: V heavy chain 5 (VH5) gene sequence

```

E V Q L V Q S G A E V K K P G E S
MfeI
~~~~~
GAAGTGCAAT TGGTTCAGAG CGGCGCGGAA GTGAAAAAAC CGGGCGAAAG
CTTCACGTTA ACCAAGTCTC GCGCGGCCTT CACTTTTTC GCGCGCTTC

L K I S C K G S G Y S F T S Y W
BspEI
~~~~~
CCTGAAAATT AGCTGCAAAG GTTCCGGATA TTCCTTTACG AGCTATTGGA
GGACTTTTAA TCGACGTTTC CAAGGCCTAT AAGGAAATGC TCGATAACCT

I G W V R Q M P G K G L E W M G I
BstXI XhoI
~~~~~
TTGGCTGGGT GCGCCAGATG CCTGGGAAGG GTCTCGAGTG GATGGGCATT
AACCGACCCA CGCGGTCTAC GGACCCTTCC CAGAGCTCAC CTACCCGTAA

I Y P G D S D T R Y S P S F Q G Q
ATTATCCGG GCGATAGCGA TACCCGTTAT TCTCCGAGCT TTCAGGGCCA
TAAATAGGCC CGCTATCGCT ATGGGCAATA AGAGGCTCGA AAGTCCCGGT

```

Figure 5F: V heavy chain 5 (VH5) gene sequence (continued)

```

V   T   I   S   A   D   K   S   I   S   T   A   Y   L   Q   W
BstEII
~~~~~
GGTGACCATT AGCGCGGATA AAAGCATTAG CACCGCGTAT CTCAATGGA
CCACTGGTAA TCGGCGCTAT TTCGTAATC GTGGCGCATA GAAGTTACCT

S   S   L   K   A   S   D   T   A   M   Y   Y   C   A   R   W   G
                               BSSHII
                               ~~~~~
GCAGCCTGAA AGCGAGCGAT ACGGCCATGT ATTATTGCGC GCGTTGGGGC
CGTCGGAATT TCGCTCGCTA TGCCGGTACA TAATAACGCG CGCAACCCCG

G   D   G   F   Y   A   M   D   Y   W   G   Q   G   T   L   V   T
                               StyI
                               ~~~~~
GGCGATGGCT TTTATGCCGAT GGATTATTGG GGCCAAGGCA CCCTGGTGAC
CCGCTACCGA AAATACGCTA CCTAATAACC CCGGTTCCGT GGGACCCACTG

V   S   S
      BspI
      ~~~~~
GGTAGCTCA G
CCAATCGAGT C

```

Figure 5G: V heavy chain 6 (VH6) gene sequence

```

Q V Q L Q Q Q S G P G L V K P S Q T
MfeI
~~~~~
CAGGTGCAAT TGCAACAGTC TGGTCCGGGC CTGGTGAAAC CGAGCCAAAC
GTCCACGTTA ACGTGTGTCAG ACCAGGCCCG GACCACTTTG GCTCGGTTTG

L S L T C A I S G D S V S S N S
BspEI
~~~~~
CCTGAGCCTG ACCTGTGCGA TTCCCGGAGA TAGCGTGAGC AGCAACAGCG
GACTCGGAC TGGACACGCT AAAGGCCTCT ATCGCACTCG TCGTTGTGCG

A A W N W I R Q S P G R G L E W L
BstXI
~~~~~
XhoI
~~~~~
CGGCGTGGAA CTGGATTCGC CAGTCTCCTG GGCGTGGCCT CGAGTGGCTG
GCCGCACCTT GACCTAAGCG GTCAGAGGAC CCGCACCGGA GTCACCCGAC

G R T Y Y R S K W Y N D Y A V S V
GGCGTACCT ATTATCGTAG CAAATGGTAT AACGATTATG CCGTGAGCGT
CCGGCATGGA TAATAGCATC GTTTACCATA TTGCTAATAC GCCACTCGCA

```

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Figure 5G: V heavy chain 6 (VH6) gene sequence (continued)

```

K  S  R  I  T  I  N  P  D  T  S  K  N  Q  F  S
      BsaBI      NspV
      ~~~~~
GAAAGCCGG ATTACCATCA ACCCGGATAC TTCGAAAAC CAGTTAGCC
CTTTTCGGCC TAATGGTAGT TGGCCCTATG AAGCTTTTG GTCAAAATCGG

L  Q  L  N  S  V  T  P  E  D  T  A  V  Y  Y  C  A
      EagI      BssHII
      ~~~~~
TGCAACTGAA CAGCGTGACC CCGGAAGATA CGGCCGTGTA TTATTGCGCG
ACGTGACTT GTCGCACTGG GGCCTTCTAT GCCGGCACAT AATAACGCGC

R  W  G  G  D  G  F  Y  A  M  D  Y  W  G  Q  G  T
      BssHII      StyI
      ~~~~~
CGTTGGGGCG GCGATGGCTT TTATGCGATG GATTATTGGG GCCAAGGCAC
GCAACCCCGC CGTACCGAA AATACGCTAC CTAATAACCC CGGTTCCGTG

L  V  T  V  S  S
      BlnI
      ~~~~~
CCTGGTGACG GTAGCTCAG
GGACCACTGC CAATCGAGTC

```

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Figure 6: oligonucleotides for gene synthesis

O1K1 5' - GAATGCATACGCTGATATCCAGATGACCCAGAG-  
CCCGTCTAGCCTGAGC -3'

O1K2 5' - CGCTCTGCAGGTAATGGTCACACGATCACCCAC-  
GCTCGCGCTCAGGCTAGACGGGC -3'

O1K3 5' - GACCATTACCTGCAGAGCGAGCCAGGGCATTAG-  
CAGCTATCTGGCGTGGTACCAGCAG -3'

O1K4 5' - CTTTGCAAGCTGCTGGCTGCATAAATTAATAGT-  
TTCGGTGCTTTACCTGGTTTCTGCTGGTACCACGCCAG -3'

O1K5 5' - CAGCCAGCAGCTTGCAAAGCGGGGTCCCGTCCC-  
GTTTTAGCGGCTCTGGATCCGGCACTGATTTTAC -3'

O1K6 5' - GATAATAGGTCGCAAAGTCTTCAGGTTGCAGGC-  
TGCTAATGGTCAGGGTAAAATCAGTGCCGGATCC -3'

O2K1 5' - CGATATCGTGATGACCCAGAGCCCACTGAGCCT-  
GCCAGTGACTCCGGGCGAGCC -3'

O2K2 5' - GCCGTTGCTATGCAGCAGGCTTTGGCTGCTTCT-  
GCAGCTAATGCTCGCAGGCTCGCCCGGAGTCAC -3'

O2K3 5' - CTGCTGCATAGCAACGGCTATAACTATCTGGAT-  
TGGTACCTTCAAAAACCAGGTCAAAGCCC -3'

O2K4 5' - CGATCCGGGACCCCACTGGCACGGTTGCTGCCC-  
AGATAAATTAATAGCTGCGGGCTTTGACCTGGTTTTTG -3'

O2K5 5' - AGTGGGGTCCCGGATCGTTTTAGCGGCTCTGGA-  
TCCGGCACCGATTTTACCCTGAAAATTAGCCGTGTG -3'

O2K6 5' - CCATGCAATAATACAGCCACGTCTTCAGCTT-  
CCACACGGCTAATTTTCAGGG -3'

O3K1 5' - GAATGCATACGCTGATATCGTGCTGACCCAGAG-  
CCCGG -3'

O3K2 5' - CGCTCTGCAGCTCAGGGTCGCACGTTGCCCCG-  
AGACAGGCTCAGGGTCGCCGGGCTCTGGGTCAGC -3'

O3K3 5' - CCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCA-  
GCAGCTATCTGGCGTGGTACCAG -3'

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Figure 6: (continued)

O3K4 5' - GCACGGCTGCTCGCGCCATAAATTAATAGACGC-  
GGTGCTTGACCTGGTTTCTGCTGGTACCACGCCAGATAG -3'

O3K5 5' - GCGCGAGCAGCCGTGCAACTGGGGTCCCCGGCGC-  
GTTTTAGCGGCTCTGGATCCGGCACGGATTTTAC -3'

O3K6 5' - GATAATACACCGCAAAGTCTTCAGGTTCCAGGC-  
TGCTAATGGTCAGGGTAAAATCCGTGCCGGATC -3'

O4K1 5' - GAATGCATACGCTGATATCGTGATGACCCAGAG-  
CCCGGATAGCCTGGCG -3'

O4K2 5' - GCTTCTGCAGTTAATGGTTCGCACGTTTCGCCAG-  
GCTCACCGCCAGGCTATCCGGGC -3'

O4K3 5' - CGACCATTAAGTGCAGAAGCAGCCAGAGCGTGC-  
TGTATAGCAGCAACAACAAAACCTATCTGGCGTGGTACCAG -  
3'

O4K4 5' - GATGCCCAATAAATTAATAGTTTCGGCGGCTGA-  
CCTGGTTTCTGCTGGTACCACGCCAGATAG -3'

O4K5 5' - AAACCTATTAATTTATTGGGCATCCACCCGTGAA-  
AGCGGGGTCCCGGATCGTTTTAGCGGCTCTGGATCCGGCAC-  
3'

O4K6 5' - GATAATACACCGCCACGTCTTCAGCTTGCAGGG-  
ACGAAATGGTCAGGGTAAAATCAGTGCCGGATCCAGAGCC -  
3'

O1L1 5' - GAATGCATACGCTCAGAGCGTGCTGACCCAGCC-  
GCCTTCAGTGAGTGG -3'

O1L2 5' - CAATGTTGCTGCTGCTGCCGCTACACGAGATGG-  
TCACACGCTGACCTGGTGCGCCACTCACTGAAGGCGGC -3'

O1L3 5' - GGCAGCAGCAGCAACATTGGCAGCAACTATGTG-  
AGCTGGTACCAGCAGTTGCCCGGGAC -3'

O1L4 5' - CCGGCACGCCTGAGGGACGCTGGTTGTTATCAT-  
AAATCAGCAGTTTCGGCGCCGTCCCGGGCAACTGC -3'

O1L5 5' - CCCTCAGGCGTGCCGGATCGTTTTAGCGGATCC-  
AAAAGCGGCACCAGCGCGAGCCTTGCG -3'

Figure 6: (continued)

O1L6 5' - CCGCTTCGTCTTCGCTTTGCAGGCCCGTAATCG-  
CAAGGCTCGCGCTGG -3'

O2L1 5' - GAATGCATACGCTCAGAGCGCACTGACCCAGCC-  
AGCTTCAGTGAGCGGC -3'

O2L2 5' - CGCTGCTAGTACCCGTACACGAGATGGTAATGC-  
TCTGACCTGGTGAGCCGCTCACTGAAGCTGG -3'

O2L3 5' - GTACGGGTACTAGCAGCGATGTGGGCGGCTATA-  
ACTATGTGAGCTGGTACCAGCAGCATCCCCG -3'

O2L4 5' - CGCCTGAGGGACGGTTGCTCACATCATAAATCA-  
TCAGTTTCGGCGCCTTCCCCGGGATGCTGCTGGTAC -3'

O2L5 5' - CAACCGTCCCTCAGGCGTGAGCAACCGTTTTCAG-  
CGGATCCAAAAGCGGCAACACCGCGAGCC -3'

O2L6 5' - CCGCTTCGTCTTCCGCTTGCAGGCCGCTAATGG-  
TCAGGCTCGCGGTGTTGCCG -3'

O3L1 5' - GAATGCATACGCTAGCTATGAACTGACCCAGCC-  
GCCTTCAGTGAGCG -3'

O3L2 5' - CGCCCAGCGCATCGCCGCTACACGAGATACGCG-  
CGGTCTGACCTGGTGCAACGCTCACTGAAGGCGGC -3'

O3L3 5' - GGCGATGCGCTGGGCGATAAATACGCGAGCTGG-  
TACCAGCAGAAACCCGGGCAGGCGC -3'

O3L4 5' - GCGTTCCGGGATGCCTGAGGGACGGTCAGAATC-  
ATCATAAATCACCAGAACTGGCGCCTGCCCCGGGTTTC -3'

O3L5 5' - CAGGCATCCCGGAACGCTTTAGCGGATCCAACA-  
GCGGCAACACCGCGACCCTGACCATTAGCGG -3'

O3L6 5' - CCGCTTCGTCTTCCGCCTGAGTGCCGCTAATGG-  
TCAGGGTC -3'

O1246H1 5' - GCTCTTCACCCCTGTTACCAAAGCCCAG-  
GTGCAATTG -3'

O1AH2 5' - GGCTTTGCAGCTCACTTTCACGCTGCTGCCCCG-  
TTTTTTCACCTCCGCGCCAGACTGAACCAATTGCACCTGGGC-  
TTTG -3'

Figure 6: (continued)

**O1AH3** 5' - GAAAGTGAGCTGCAAAGCCTCCGGAGGCACTTT-  
TAGCAGCTATGCGATTAGCTGGGTGCGCCAAGCCCCTGGGCAG  
GGTC -3'

**O1AH4** 5' - GCCCTGAAACTTCTGCGCGTAGTTCGCCGTGCC-  
AAAAATCGGAATAATGCCGCCCATCCACTCGAGACCCTGCCC-  
AGGGGC -3'

**O1AH5** 5' - GCGCAGAAGTTTCAGGGCCGGGTGACCATTACC-  
GCGGATGAAAGCACCAGCACCGCGTATATGGAAGTGAAGCAGCC  
TGCG -3'

**O1ABH6** 5' - GCGCGCAATAATACACGGCCGTATCTTCGCT-  
ACGCAGGCTGCTCAGTTCC -3'

**O1BH2** 5' - GGCTTTGCAGCTCACTTTCACGCTCGCGCCCGG-  
TTTTTTCACTTCCGCGCCGCTCTGAACCAATTGCACCTGGGC-  
TTTG -3'

**O1BH3** 5' - GAAAGTGAGCTGCAAAGCCTCCGGATATACCTT-  
TACCAGCTATTATATGCACTGGGTCCGCCAAGCCCCTGGGCAG  
GGTC -3'

**O1BH4** 5' - GCCCTGAAACTTCTGCGCGTAGTTCGTGCCGCC-  
GCTATTCGGGTTAATCCAGCCCATCCACTCGAGACCCTGCCCCA  
GGGGC -3'

**O1BH5** 5' - GCGCAGAAGTTTCAGGGCCGGGTGACCATGACC-  
CGTGATACCAGCATTAGCACCGCGTATATGGAAGTGAAGCAGCC  
TGCG -3'

**O2H2** 5' - GGTACAGGTCAGGGTCAGGGTTTGGGTGCGTTT-  
CACCAGGGCCGGGCGCTTTCTTTCAATTGCACCTGGGCTTTG  
-3'

**O2H3** 5' - CTGACCCTGACCTGTACCTTTTCCGGATTTAGC-  
CTGTCCACGTCTGGCGTTGGCGTGGGCTGGATTGCCAGCCGC  
CTGGGAAAG -3'

**O2H4** 5' - GCGTTTTTCAGGCTGGTGCTATAATACTTATCAT-  
CATCCCAATCAATCAGAGCCAGCCACTCGAGGGCTTTCCCAGG  
CGGCTGG -3'

Figure 6: (continued)

**02H5** 5' - GCACCAGCCTGAAAACGCGTCTGACCATTAGCA-  
AAGATACTTCGAAAAATCAGGTGGTGCTGACTATGACCAACAT  
GG -3'

**02H6** 5' - GCGCGCAATAATAGGTGGCCGTATCCACCGGGT-  
CCATGTTGGTCATAGTCAGC -3'

**03H1** 5' - CGAAGTGCAATTGGTGGAAAGCGGCGGCCT-  
GGTGCAACCGGGCGGCAG -3'

**03H2** 5' - CATAGCTGCTAAAGGTAAATCCGGAGGCCGCGC-  
AGCTCAGACGCAGGCTGCCGCCCGGTTGCAC -3'

**03H3** 5' - GATTTACCTTTAGCAGCTATGCGATGAGCTGGG-  
TGCGCCAAGCCCCTGGGAAGGGTCTCGAGTGGGTGAG -3'

**03H4** 5' - GGCCTTTCACGCTATCCGCATAATAGGTGCTGC-  
CGCCGCTACCGCTAATCGCGCTCACCCACTCGAGACCC -3'

**03H5** 5' - CGGATAGCGTGAAAGGCCGTTTTACCATTTAC-  
GTGATAATTCGAAAAACACCCTGTATCTGCAAATGAACAG-3'

**03H6** 5' - CACGCGCGCAATAATACACGGCCGTATCTTCCG-  
CACGCAGGCTGTTTCATTTGCAGATACAGG -3'

**04H2** 5' - GGTCAGGCTCAGGGTTTCGCTCGGTTTCACCAG-  
GCCCCGGACCACTTTCTTGCAATTGCACCTGGGCTTTG -3'

**04H3** 5' - GAAACCCTGAGCCTGACCTGCACCGTTTCCGGA-  
GGCAGCATTAGCAGCTATTATTGGAGCTGGATTGCCAGCCGC  
-3'

**04H4** 5' - GATTATAGTTGGTGCTGCCGCTATAATAAATAT-  
AGCCAATCCACTCGAGACCCTTCCCAGGCGGCTGGCGAATCCA  
G -3'

**04H5** 5' - CGGCAGCACCAACTATAATCCGAGCCTGAAAAG-  
CCGGGTGACCATTAGCGTTGATACTTCGAAAAACCAGTTTAGC  
CTG -3'

**04H6** 5' - GCGCGCAATAATACACGGCCGTATCCGCCGCCG-  
TCACGCTGCTCAGTTTCAGGCTAAACTGGTTTTTCG -3'

Figure 6: (continued)

**O5H1** 5' - GCTCTTCACCCCTGTTACCAAAGCCGAAGTGCA-  
ATTG -3'

**O5H2** 5' - CCTTTGCAGCTAATTTTCAGGCTTTCGCCCCGGT-  
TTTTTCACTTCCGCGCCGCTCTGAACCAATTGCACTTCGGCTT  
TGG -3'

**O5H3** 5' - CCTGAAAATTAGCTGCAAAGGTTCCGGATATTC-  
CTTTACGAGCTATTGGATTGGCTGGGTGCGCCAGATGCCTGG  
-3'

**O5H4** 5' - CGGAGAATAACGGGTATCGCTATCGCCCCGGATA-  
AATAATGCCCATCCACTCGAGACCCTTCCCAGGCATCTGGCGC  
AC -3'

**O5H5** 5' - CGATACCCGTTATTCTCCGAGCTTTCAGGGCCA-  
GGTGACCATTAGCGCGGATAAAAGCATTAGCACCGCGTATCTT  
C -3'

**O5H6** 5' - GCGCGCAATAATACATGGCCGTATCGCTCGCTT-  
TCAGGCTGCTCCATTGAAGATACGCGGTGCTAATG -3'

**O6H2** 5' - GAAATCGCACAGGTCAGGCTCAGGGTTTGGCTC-  
GGTTTCACCAGGCCCGGACCAGACTGTTGCAATTGCACCTGG-  
GCTTTG -3'

**O6H3** 5' - GCCTGACCTGTGCGATTTCCGGAGATAGCGTGA-  
GCAGCAACAGCGCGGCGTGGAAGTGGATTCGCCAGTCTCCTGG  
GCG -3'

**O6H4** 5' - CACCGCATAATCGTTATACCATTTGCTACGATA-  
ATAGGTACGGCCCAGCCACTCGAGGCCACGCCCAGGAGACTG-  
GCG -3'

**O6H5** 5' - GGTATAACGATTATGCGGTGAGCGTGAAAAGCC-  
GGATTACCATCAACCCGGATACTTCGAAAAACCAGTTTAGCCT  
GC -3'

**O6H6** 5' - GCGCGCAATAATACACGGCCGTATCTTCCGGGG-  
TCACGCTGTTCAAGTTCAGGCTAAACTGGTTTTTC -3'

**OCLK1** 5' - GGCTGAAGACGTGGGCGTGTATTATTGCCAGCA-  
GCATTATACCACCCCGCCGACCTTTGGCCAGGGTAC -3'

Figure 6: (continued)

OCLK2 5' - GCGGAAAAATAAACACGCTCGGAGCAGCCACCG-  
TACGTTTAATTTCAACTTTTCGTACCCTGGCCAAAGGTC -3'

OCLK3 5' - GAGCGTGTTTATTTTTCCGCCGAGCGATGAACA-  
ACTGAAAAGCGGCACGGCGAGCGTGGTGTGCCTGCTG -3'

OCLK4 5' - CAGCGCGTTGTCTACTTTCCACTGAACTTTCGC-  
TTCACGCGGATAAAAGTTGTTCAGCAGGCACACCACGC -3'

OCLK5 5' - GAAAGTAGACAACGCGCTGCAAAGCGGCAACAG-  
CCAGGAAAGCGTGACCGAACAGGATAGCAAAGATAG -3'

OCLK6 5' - GTTTTTTCATAATCCGCTTTGCTCAGGGTCAGGG-  
TGCTGCTCAGAGAATAGGTGCTATCTTTGCTATCCTGTTGC -  
3'

OCLK7 5' - GCAAAGCGGATTATGAAAAACATAAAGTGTATG-  
CGTGCGAAGTGACCCATCAAGGTCTGAGCAGCCCGGTG -3'

OCLK8 5' - GGCATGCTTATCAGGCCTCGCCACGATTAAAAG-  
ATTTAGTCACCGGGCTGCTCAGAC -3'

OCH1 5' - GGCGTCTAGAGGCCAAGGCACCCTGGTGACGGT-  
TAGCTCAGCGTCGAC -3'

OCH2 5' - GTGCTTTTGCTGCTCGGAGCCAGCGGAAACACG-  
CTTGGACCTTTGGTCGACGCTGAGCTAACC -3'

OCH3 5' - CTCCGAGCAGCAAAAGCACCAGCGGCGGCACGG-  
CTGCCCTGGGCTGCCTGGTTAAAGATTATTTCC -3'

OCH4 5' - CTGGTCAGCGCCCCGCTGTTCCAGCTCACGGTG-  
ACTGGTTCCGGGAAATAATCTTTAACCAGGCA -3'

OCH5 5' - AGCGGGGCGCTGACCAGCGGCGTGCATACCTTT-  
CCGGCGGTGCTGCAAAGCAGCGGCCTG -3'

OCH6 5' - GTGCCTAAGCTGCTGCTCGGCACGGTCACAACG-  
CTGCTCAGGCTATACAGGCCGCTGCTTTGCAG -3'

OCH7 5' - GAGCAGCAGCTTAGGCACTCAGACCTATATTTG-  
CAACGTGAACCATAAACCAGCAACACC -3'

OCH8 5' - GCGCGAATTCGCTTTTCGGTTCCACTTTTTTAT-  
CCACTTTGGTGTTGCTCGGTTTATGG -3'

Figure 7A: sequence of the synthetic Cx gene segment

```

      * V  A  A  A  P  S  V  F  I  F  P  P  S  D  E  Q
      BsiWI
      ~~~~~
CGTACGGTGG CTGCTCCGAG CGTGTTTATT TTTCCGCCGA GCGATGAACA
GCATGCCACC GACGAGGCTC GCACAAATAA AAAGGCCGGCT CGCTACTTGT

      L  K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y
ACTGAAAAGC GGCACGGCGA GCGTGGTGTG CCTGCTGAAC AACTTTTATC
TGACTTTTCG CCGTGCCGCT CGCACCCACAC GGACGACTTG TTGAAAATAG

      P  R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  G
CGCGTGAAGC GAAAGTTCAG TGGAAAGTAG ACAACGCGCT GCAAAGCGGC
GCGCACTTCG CTTCAAGTC ACCTTTCATC TGTGCGCGA CGTTTCGCCG

      N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S
AACAGCCAGG AAAGCGTGAC CGAACAGGAT AGCAAAGATA GCACCTATTC
TTGTCCGTCC TTTCGCACTG GCTTGTCCTA TCGTTTCTAT CGTGGATAAG

      L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K
TCTGAGCAGC ACCCTGACCC TGAGCAAAGC GGATTATGAA AAACATAAAG
AGACTCGTCG TGGGACTGGG ACTCGTTTCG CCTAATACTT TTTGTATTTC

```



Figure 7A: sequence of the synthetic Cκ gene segment (continued)

V Y A C E V T H Q G L S S P V T K  
 TGTATGCGTG CGAAGTGACC CATCAAGGTC TGAGCAGCCCC GTGACTAAA  
 ACATACGCAC GCTTCACTGG GTAGTCCAG ACTCGTCGGG CCACTGATTT

S F N R G E A \*

StuI SphI

-----  
 TCTTTTAATC GTGGCGAGGC CTGATAAGCA TGC  
 AGAAAATTAG CACCGCTCCG GACTATTTCGT ACG

Figure 78: sequence of the synthetic CH1 gene segment

```

      A  S  T  K  G  P  S  V  F  P  L  A  P  S  S
BlpI  Sali
~~~~~
GCTCAGCGTC GACCAAAGGT CCAAGCGTGT TTCCGCTGGC TCCGAGCAGC
CGAGTCGCAG CTGGTTTCCA GGTTCGCACA AAGGCGACCG AGGCTCGTCG

      K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y
AAAAGCACCA GCGCGGCAC GGCTGCCCTG GGCTGCCCTGG TTAAGATTA
TTTTCGTGGT CGCCGCCGTG CCGACGGGAC CCGACGGACC AATTCTAAT

      F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S
TTTCCCGGAA CCAGTCACCG TGAGCTGGAA CAGCGGGGCG CTGACCAGCG
AAAGGGCCTT GGTCA GTGC ACTCGACCTT GTCGCCCCCG GACTGGTCGC

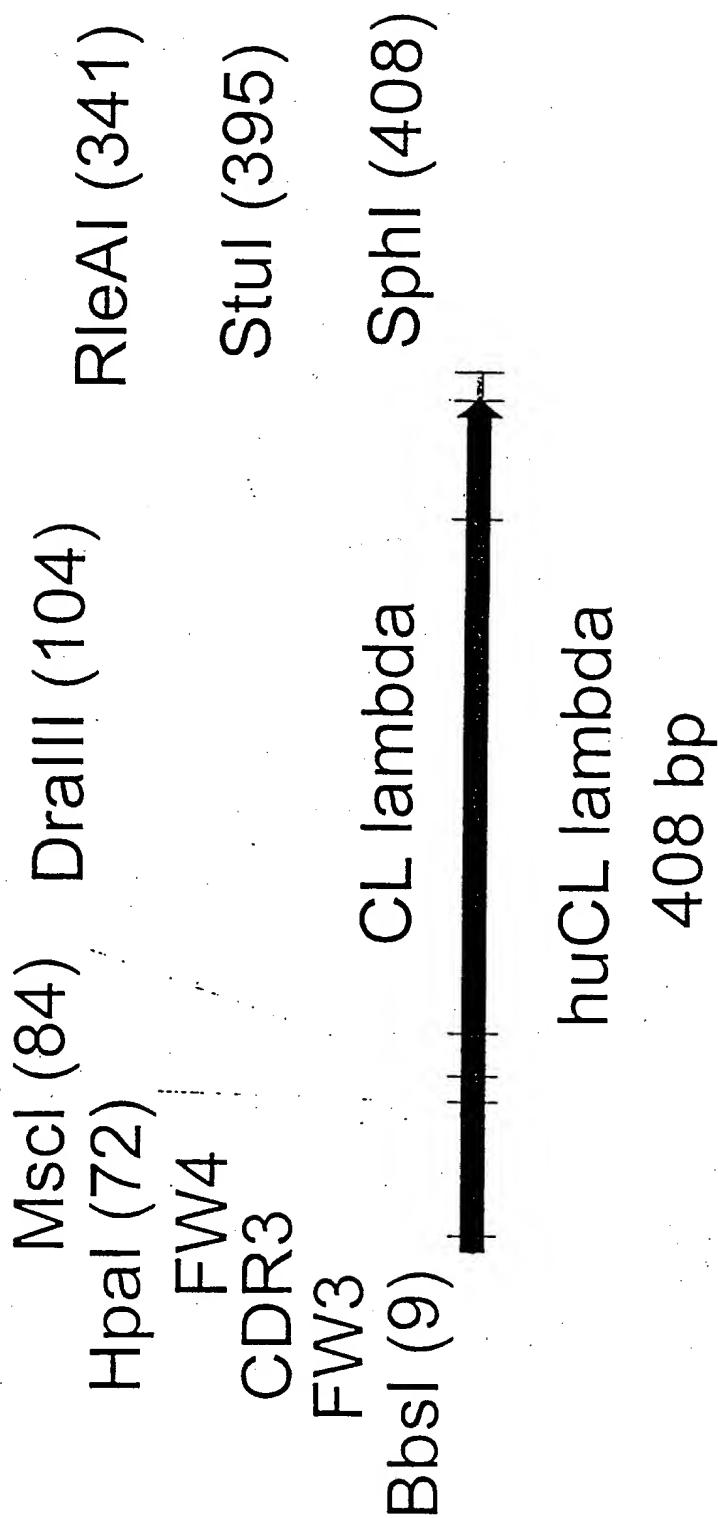
      G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L
GCGTGATAC CTTCCGGCG GTGCTGCAAA GCAGCGGCCT GTATAGCCTG
CGCACGTATG GAAAGGCCCG CACGACGTTT CGTCGCCGGA CATATCGGAC

      S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I
AGCAGCGTTG TGACCGTGCC GAGCAGCAGC TTAGGCACTC AGACCTATAT
TCGTCGCAAC ACTGGCACGG CTCGTCGTCG AATCCGTGAG TCTGGATATA

```

Figure 7B: sequence of the synthetic CH1 gene segment (continued)

|            |            |            |            |            |   |         |   |   |   |   |   |   |   |   |   |
|------------|------------|------------|------------|------------|---|---------|---|---|---|---|---|---|---|---|---|
| C          | N          | V          | N          | H          | K | P       | S | N | T | K | V | D | K | K | V |
| TTGCAACGTG | AACCATAAAC | CGAGCAACAC | CAAAGTGGAT | AAAAAAGTGG |   |         |   |   |   |   |   |   |   |   |   |
| AACGTTGCAC | TTGGTATTG  | GCTCGTTGTG | GTTTCACCTA | TTTTTTCACC |   |         |   |   |   |   |   |   |   |   |   |
| E          | P          | K          | S          | E          | F | *       |   |   |   |   |   |   |   |   |   |
|            |            |            |            | EcoRI      |   | HindIII |   |   |   |   |   |   |   |   |   |
|            |            |            |            | ~~~~~      |   | ~~~~~   |   |   |   |   |   |   |   |   |   |
| AACCGAAAAG | CGAATTCTGA | TAAGCTT    |            |            |   |         |   |   |   |   |   |   |   |   |   |
| TTGGCTTTC  | GCTTAAGACT | ATTCGAA    |            |            |   |         |   |   |   |   |   |   |   |   |   |

Figure 7C: functional map and sequence of module 24 comprising the synthetic C $\lambda$  gene segment (huCL lambda)

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Figure 7C: functional map and sequence of module 24 comprising the synthetic CI gene segment (huCI lambda) (continued)

| Bbs I   |  | Hpa I |       | Msc I |       | Dra III |       |
|---------|--|-------|-------|-------|-------|---------|-------|
|         | ~~~~~  |       | ~~~~~ |       | ~~~~~ |         | ~~~~~ |
| 1       | GAAGACGAAG CGATTATTA TTGCCAGCAG CATTATACCA CCCCGCCTGT    |       |       |       |       |         |       |
|         | CTTCTGCTTC GCCTAATAAT AACGGTCGTC GTAATATGGT GGGCGGGACA   |       |       |       |       |         |       |
| 51      | GTTTGGCGGC GGCACGAAGT TAACCGTTCT TGGCCAGCCG AAAGCCGCAC   |       |       |       |       |         |       |
|         | CAAACCGCCG CCGTGCTTCA ATGGCAAGA ACCGGTCGGC TTTCGGCGTG    |       |       |       |       |         |       |
| Dra III |  | Hpa I |       | Msc I |       | Dra III |       |
|         | ~~~~~  |       | ~~~~~ |       | ~~~~~ |         | ~~~~~ |
| 101     | CGAGTGTGAC GCTGTTTCCG CCGAGCAGCG AAGAATTGCA GCGAACAATA   |       |       |       |       |         |       |
|         | GCTCACACTG CGACAAAGGC GGCTCGTCGC TTCTTAACGT CCGCTTGTTT   |       |       |       |       |         |       |
| 151     | GCGACCCCTGG TGTGCCTGAT TAGCGACTTT TATCCGGGGAG CCGTGACAGT |       |       |       |       |         |       |
|         | CGCTGGGACC ACACGGACTA ATCGCTGAAA ATAGGCCCTC GGCACGTCA    |       |       |       |       |         |       |
| 201     | GGCCTGGAAG GCAGATAGCA GCCCCGTCAA GGCGGGAGTG GAGACCACCA   |       |       |       |       |         |       |
|         | CCGGACCTTC CGTCTATCGT CGGGGCAGTT CCGCCCTCAC CTCTGGTGGT   |       |       |       |       |         |       |

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Figure 7C: functional map and sequence of module 24 comprising the synthetic Cl gene segment (huCl lambda) (continued)

251 CACCCTCCAA ACAAGCAAC AACAAGTACG CGGCCAGCAG CTATCTGAGC  
GTGGGAGGTT TGTTTCGTTG TTGTTTCATGC GCCGGTCGTC GATAGACTCG

RleAI

~~~~~

301 CTGACGCCTG AGCAGTGGAA GTCCCACAGA AGCTACAGCT GCCAGGTCAC  
GACTGCCGGAC TCGTCACCTT CAGGGTGTCT TCGATGTCGA CGGTCCAGTG

StuI

~~~~~

351 GCATGAGGGG AGCACCGTGG AAAAAACCGT TGCGCCGACT GAGGCCCTGAT  
CGTACTCCCC TCGTGGCACC TTTTGTGGCA ACGCGGCTGA CTCCGGACTA

SphI

~~~~~

401 AAGCATGC  
TTCGTACG

Figure 7D: oligonucleotides used for synthesis of module M24 containing Cλ gene segment

M24: assembly PCR

M24-A: GAAGACAAGCGGATTATTGCCAGCAGCATTATACACCCCGCTGTGTTGGCGGCG-  
GCACGAAGTTAACCGTTC

M24-B: CAATTCCTCGCTCGCGGGAACAGCGTCACACTCGGTGCGGCTTCGGCTGGCCAA-  
GAACGGTAACTTCGTGCCGC

M24-C: CGCCGAGCAGCGAAGATTGCAGGCAACAAGCACCTGGTGTGCTGATTAGCGACT-  
TTTATCCGGGAGCCGTGACA

M24-D: TGTGGAGGGGTGGTCTCCACTCCCGCCTTGACGGGGCTGCTAICTGCCCTCCAG-  
GCCACTGTCACGGCTCCCGG

M24-E: CCACACCTCCAAACAAGCAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGC-  
CTGAGCAGTGGAAAGTCCCACAGAAGCTACAGCTG

M24-F: GCAATGCTTATCAGGCCCTCAGTCGGCGCAACGGTTTTTCCACGGTGTCTCCCCCTCATGCGT-  
GACCTGGCAGCTGTAGCTTC

Figure 8: sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vk2

```

M  K  Q  S  T  I  A  L  A  L  L  P  L  L  F  T  P
                               Sapi
                               ~~~~~
ATGAAACAAA  GCACTATTGC  ACTGGCACTC  TTACCGTTGC  TCTTACCCCC
TACTTTGTTT  CGTGATAACG  TGACCGTGAG  AATGGCAACG  AGAAGTGGGG

V  T  K  A  D  Y  K  D  E  V  Q  L  V  E  S  G
                               MfeI
                               ~~~~~
TGTTACCAAA  GCCGACTACA  AAGATGAAGT  GCAATTGGTG  GAAAGCGGCG
ACAATGGTTT  CGGCTGATGT  TTCTACTTCA  CGTTAACCCAC  CTTTCGCCCG

G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A  A  S
                               BspEI
                               ~~~~~
GCGGCCCTGGT  GCAACCGGGC  GGCAGCCTGC  GTCTGAGCTG  CGCGGCCTCC
CGCCGGACCA  CGTTGGCCCC  CCGTCGGACG  CAGACTCGAC  GCGCCGGAGG

G  F  T  F  S  S  Y  A  M  S  W  V  R  Q  A  P  G
BspEI
-----
GGATTACCT  TTAGCAGCTA  TGCATGAGC  TGGGTGCGCC  AAGCCCCCTGG
-----
CCTAAATGGA  AATCGTCGAT  ACGTACTCG  ACCCACGCGG  TTCGGGGACC

```

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Figure 8: sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vk2 (continued)

```

K G L E W V S A I S G S G S T
XhoI
~~~~~
GAAGGCTCTC GAGTGGGTGA GCGGATTAG CCGTAGCGGC GGCAGCACCT
CTTCCCAGAG CTCACCCACT CCGCTAATC GCCATCGCG CCGTCGTGGA

Y Y A D S V K G R F T I S R D N S
PmlI NspV
~~~~~
ATTATGCCGA TAGCGTGAAA GCGCGTTTTA CCATTCACG TGATAATTCTG
TAATACGCCT ATCGCACTTT CCGGCAAAAT GGTAAAGTGC ACTATTAAGC

K N T L Y L Q M N S L R A E D T A
NspV EagI
~~~
AAAAACACCC TGTATCTGCA AATGAACAGC CTGCGTGCGG AAGATACGGC
TTTTTGTTGG ACATAGACGT TTAATTGTCG GACGCACGCC TTCTATGCCG

V Y Y C A R W G G D G F Y A M D
EagI BssHII
~~~
CGTGATTAT TCGCGCGCGTT GGGCGGCGGA TGGCTTTTAT GCGATGGATT

```

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Figure 8: sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vk2 (continued)

```

GCACATAATA ACGCGCGCAA CCCGCCGCT ACCGAAAATA CGCTACCTAA
Y W G Q G T L V T V S S A G G G S
          StyI      BlnI
-----
ATTGGGGCCA AGGCACCCCTG GTGACGGTTA GCTCAGCGGG TGGCGGTTCT
TAACCCCGGT TCCGTGGGAC CACTGCCAAT CGAGTCGCC ACCGCCAAGA

G G G G S G G G G S G G G S D I
          EcoRV
-----
GGCGGCGGTG GGAGCGGTGG CCGTGGTTCT GCGGTTGGTG GTTCCGATAT
CCGCCGCCAC CCTCGCCACC GCCACCAAGA CCGCCACCAC CAAGGCTATA

V M T Q S P L S L P V T P G E P
EcoRV      BlnI
-----
CGTGATGACC CAGAGCCCCAC TGAGCCTGCC AGTGACTCCG GCGAGCCCTG
GCACTACTGG GTCTCGGGTG ACTCGGACGG TCACTGAGGC CCGCTCGGAC

A S I S C R S S Q S L L H S N G Y
          PstI
-----
CGAGCATTAG CTGCAGAAGC AGCCAAAGCC TGCTGCATAG CAACGGCTAT
GCTCGTAATC GACGTCTTCG TCGGTTTCGG ACGACGTATC GTTGCCGATA

```

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Figure 8: sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vk2 (continued)

```

N Y L D W Y L Q K P G Q S P Q L L
                                KpnI   SexAI   AseI
-----
AACTATCTGG ATTGGTACCT TCAAAAACCA GTCAAAGCC CGCAGCTATT
TTGATAGACC TAACCATGGA AGTTTTCGGT CCAGTTTCGG GCGTCGATAA

I Y L G S N R A S G V P D R F S
AseI
-----
AATTTATCTG GGCAGCAACC GTGCCAGTGG GTCCCCGGAT CGTTTTCAGC
TTAAATAGAC CCGTCGTTGG CACGGTCACC CCAGGGCCTA GCAAAATCGC

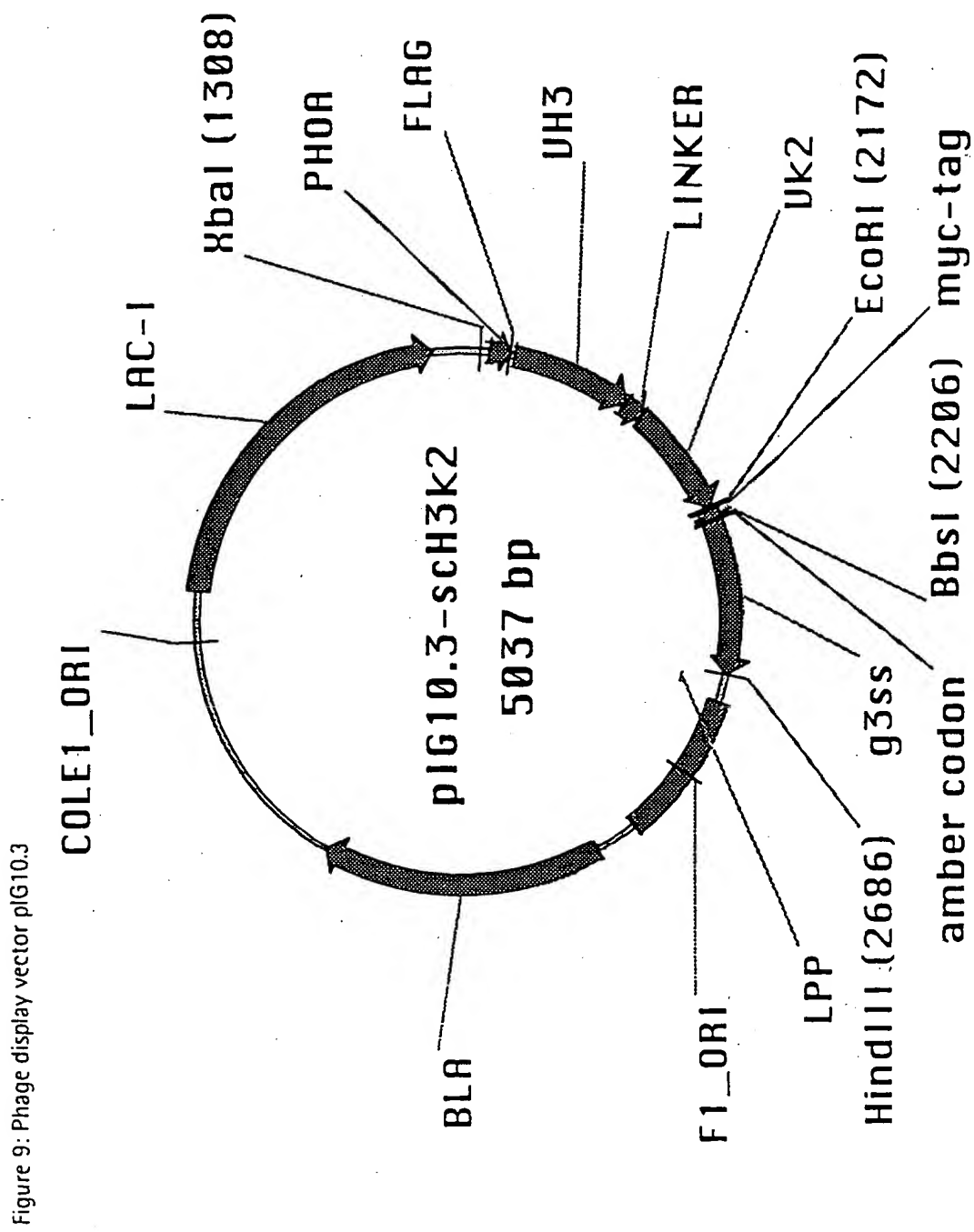
G S G S G T D F T L K I S R V E A
BamHI
-----
GCTCTGGATC CGGCACCGAT TTTACCCCTGA AAATTAGCCG TGTGGAAGCT
CGAGACCTAG GCCGTGGCTA AAATGGGACT TTTAATCGGC ACACCTTCGA

E D V G V Y Y C Q Q H Y T T P P T
BbsI
-----
GAAGACGTGG GCGTGTATTA TTGCCAGCAG CATTATACCA CCCC GCCGAC
CTTCTGCACC CGCACATAAT AACGGTCGTC GTAATATGGT GGGGCGGCTG

```

Figure 8: sequence and restriction map of the synthetic gene encoding the consensus single-chain fragment VH3-Vk2 (continued)

| F           | G          | Q          | G          | T   | K | V | E | I | K | R | T | E | F |
|-------------|------------|------------|------------|-----|---|---|---|---|---|---|---|---|---|
| MscI        |            |            |            |     |   |   |   |   |   |   |   |   |   |
| -----       |            |            |            |     |   |   |   |   |   |   |   |   |   |
| CTTTGGCCAG  | GGTACGAAAG | TTGAAATTAA | ACGTACGGAA | TTC |   |   |   |   |   |   |   |   |   |
| GAAACCGGTC  | CCATGCTTTC | AACTTTAATT | TGCATGCCTT | AAG |   |   |   |   |   |   |   |   |   |
| BsiWI ECORI |            |            |            |     |   |   |   |   |   |   |   |   |   |
| -----       |            |            |            |     |   |   |   |   |   |   |   |   |   |



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Figure 10: Sequence analysis of initial libraries

|   |      |   |   |   |   |   |   |   |   |   |   |   |   |
|---|------|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 103  | W | W | W | W | W | W | W | W | W | W | W | W |
|   | 102  | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
|   | 101  | D | D | D | D | D | D | D | D | D | D | D | D |
|   | 100E | N | - | - | - | - | - | - | - | - | - | - | - |
|   | 100D | - | - | - | - | - | - | - | - | - | - | - | - |
|   | 100C | - | - | - | - | - | - | - | - | - | - | - | - |
|   | 100B | A | - | - | - | - | - | - | - | - | - | - | - |
|   | 100A | Y | - | - | - | - | - | - | - | - | - | - | - |
|   | 100  | F | Y | H | H | R | Y | P | - | S | K | A | D |
|   | 99   | G | N | W | Y | A | G | Q | R | N | S | A | Y |
|   | 98   | D | M | E | L | K | T | A | T | R | D | F | Q |
|   | 97   | G | K | T | E | L | T | E | I | N | G | T | P |
|   | 96   | G | G | R | R | F | N | N | A | Y | V | K | A |
|   | 95   | W | F | H | V | K | W | I | T | W | S | S | V |
|   | 94   | R | R | R | R | R | R | R | R | R | R | R | R |
|   | 93   | A | A | A | A | A | A | A | A | A | A | A | A |
|   | 92   | C | C | C | C | C | C | C | C | C | C | C | C |
| B |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |
|   |      |   | C | C | C | C | C | C | C | C | C | C | C |

Figure 10: Sequence analysis of initial libraries

|   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | C | C | C | C | C | C | C | C | C | C | C | C |
| A | A | A | A | A | A | A | A | A | A | A | A | A |
| R | R | R | R | R | R | R | R | R | R | R | R | R |
| Y | M | K | T | Y | * | R | M | K | S | Y |   |   |
| F | A | N | Q | P | G | N | K | G | W | A |   |   |
| V | L | Q | S | Y | S | P | P | S | T | G |   |   |
| H | R | M | F | R | G | W | M | E | N | T |   |   |
| F | A | V | W | S | S | N | L | F | D | T |   |   |
| L | S | F | E | N | E | V | N | L | K | F |   |   |
| Y | G | H | Q | F | H | N | R | E | P | K |   |   |
| T | K | A | Q | F | W | Y | D | T | N | Q |   |   |
| M | Y | R | K | M | S | L | G | D | F | G |   |   |
| V | I | K | V | P | I | H | T | V | I | P |   |   |
| M | M | F | M | M | F | F | M | M | M | M |   |   |
| D | D | D | D | D | D | D | D | D | D | D |   |   |
| V | V | V | Y | V | V | V | V | Y | V | Y |   |   |
| W | W | W | W | W | W | W | W | W | W | W |   |   |

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Figure 11: Expression analysis of initial library

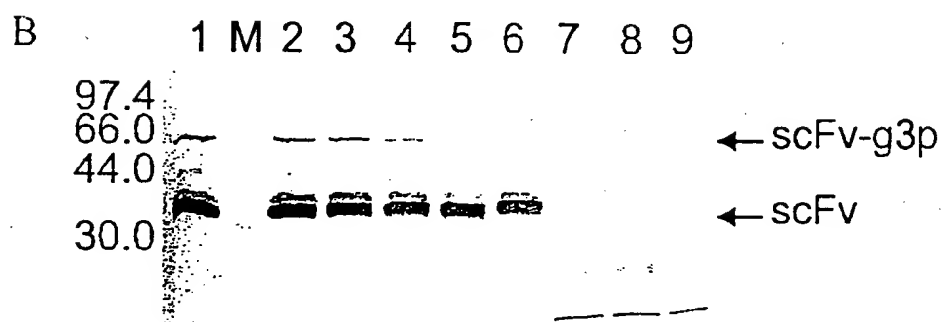
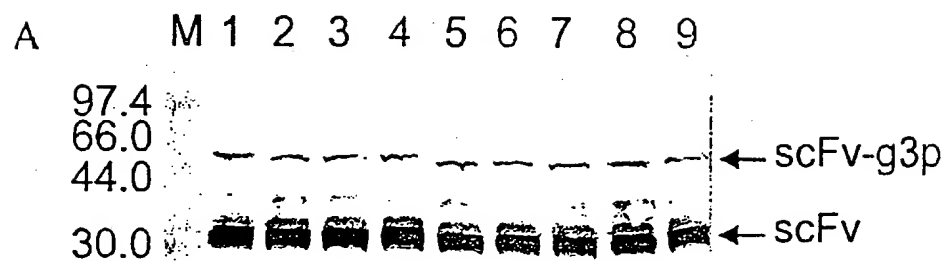




Figure 12: Increase of specificity during the panning rounds

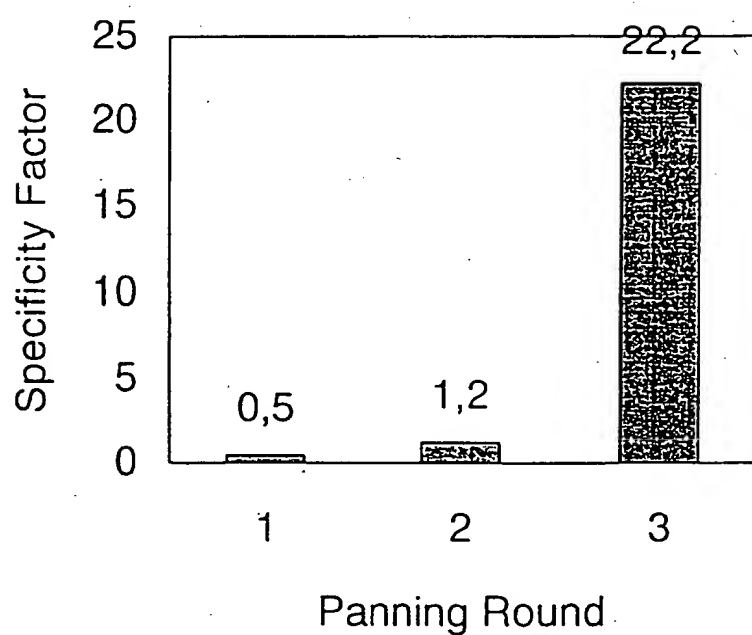


Figure 13: Phage ELISA of clones after the 3rd round of panning

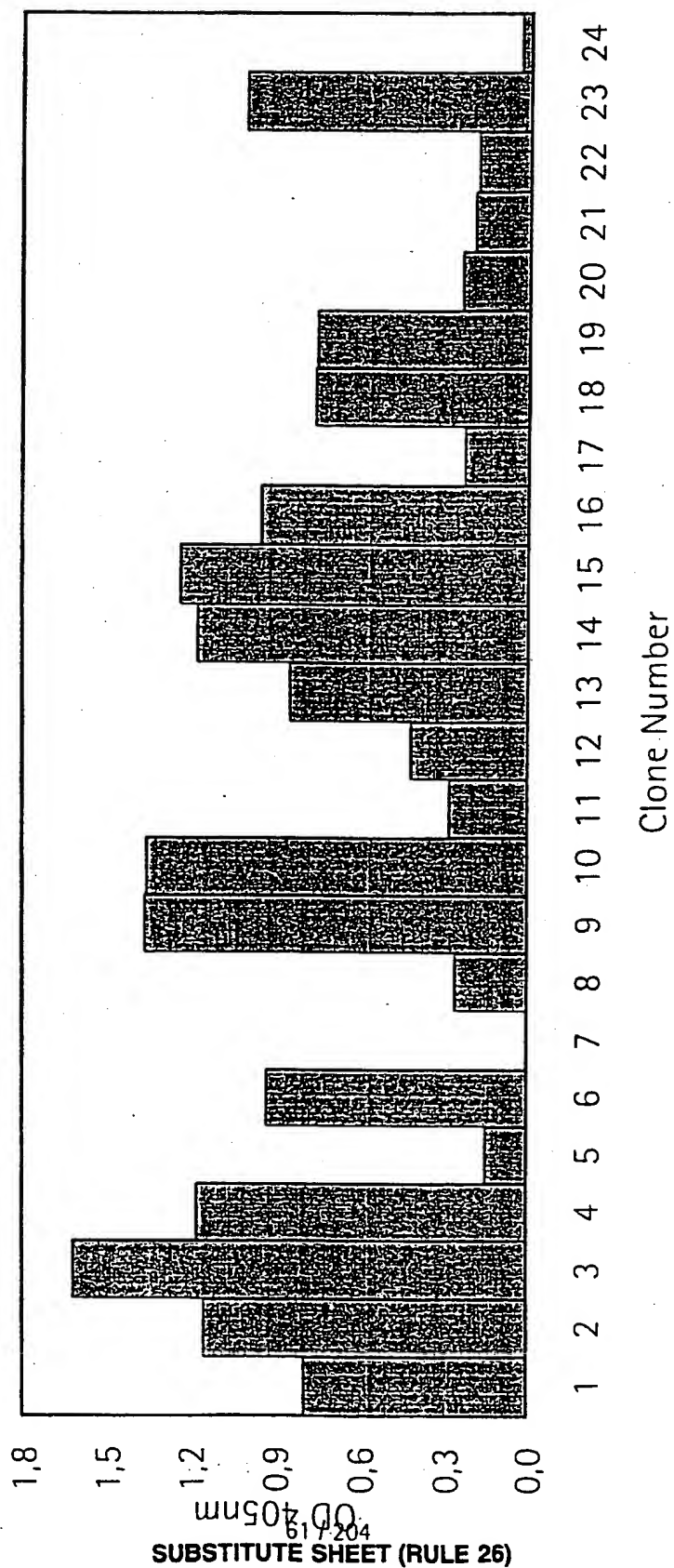


Figure 14: Competition ELISA

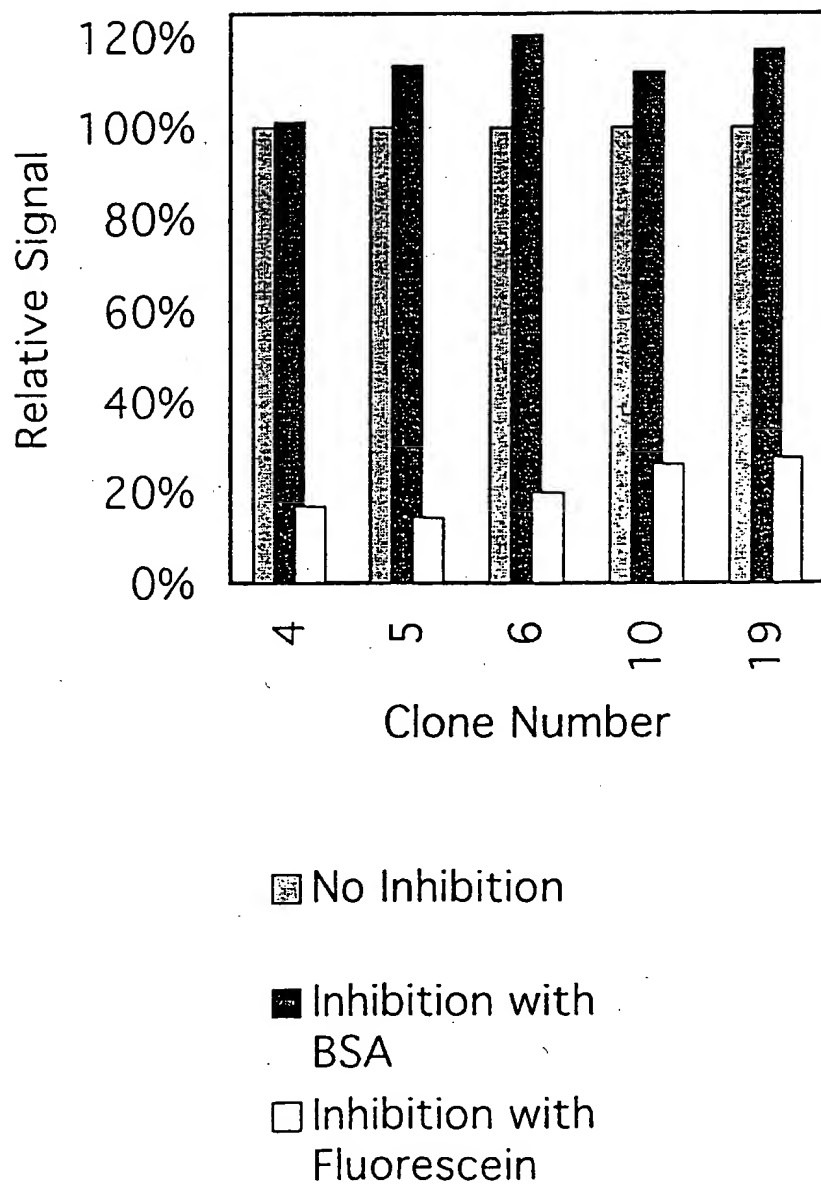


Figure 15: Sequence analysis of fluorescein binders

| Frequency | 1 | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 103       | W | W | W | W | W | W | W | W | W | W | W | W | W | W | W | W |
| 102       | > | > | > | > | > | > | > | Y | Y | > | > | > | > | > | > | Y |
| 101       | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D | D |
| 100E      | F | F | F | F | F | M | F | F | F | F | F | F | F | F | F | F |
| 100D      | R | R | R | R | S | Q | > | K | Y | R | R | R | - | Q | R | R |
| 100C      | F | R | H | R | N | D | A | > | K | D | N | P | K | K | A | S |
| 100B      | R | M | R | K | K | F | K | T | > | M | M | R | R | R | F | F |
| 100A      | P | K | L | - | W | S | K | S | R | R | R | A | K | P | S | T |
| 100       | N | R | H | R | K | P | L | Y | S | R | G | F | G | Y | R | Y |
| 99        | Q | K | R | K | M | H | F | R | R | W | R | K | K | T | R | Q |
| 98        | M | Q | K | R | - | > | M | H | M | S | R | K | H | - | K | K |
| 97        | N | K | G | M | K | E | P | F | T | R | P | K | > | H | T | L |
| 96        | R | S | N | K | R | - | K | K | K | K | N | G | M | K | W | K |
| 95        | K | R | R | R | Y | L | R | R | R | K | R | K | R | R | R | K |
| 94        | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |
| 93        | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| 92        | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C | C |

Figure 16: Purification of fluorescein binding scFv fragments

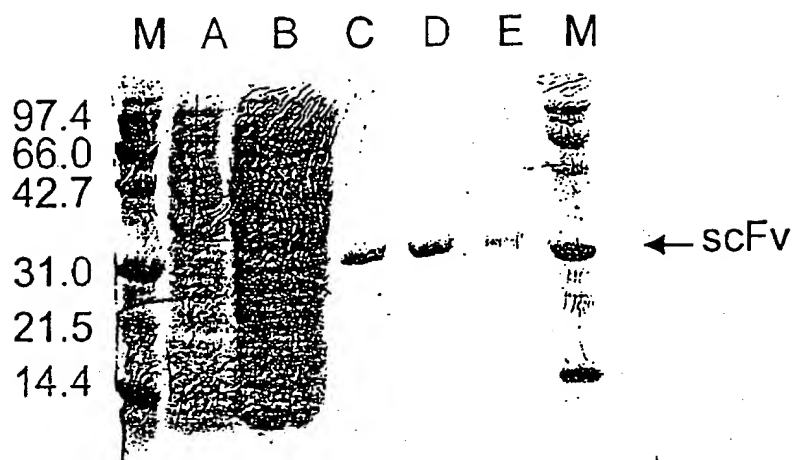


Figure 17: Enrichment factors after three rounds of panning

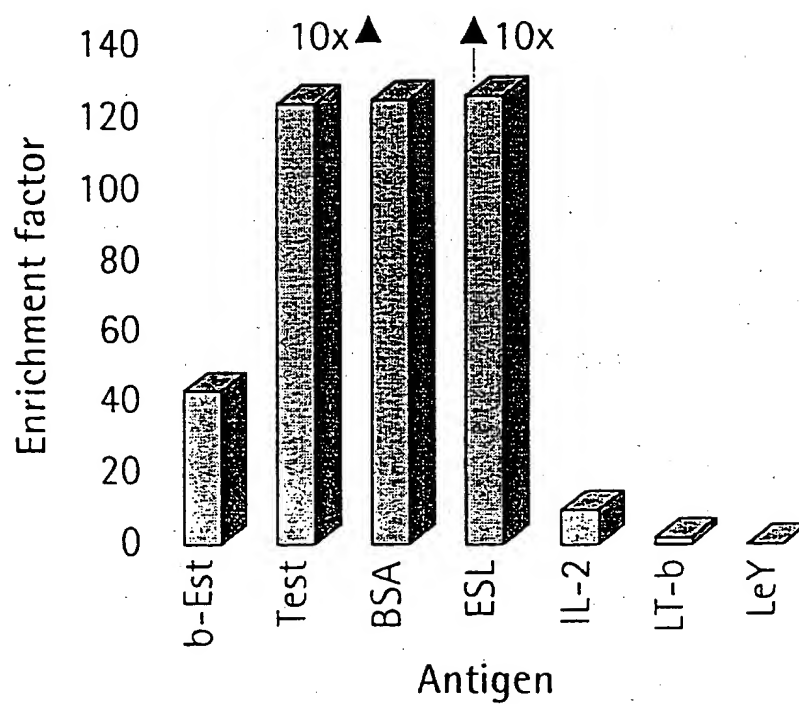


Figure 18: ELISA of anti-ESL-1 and anti- $\beta$ -estradiol antibodies

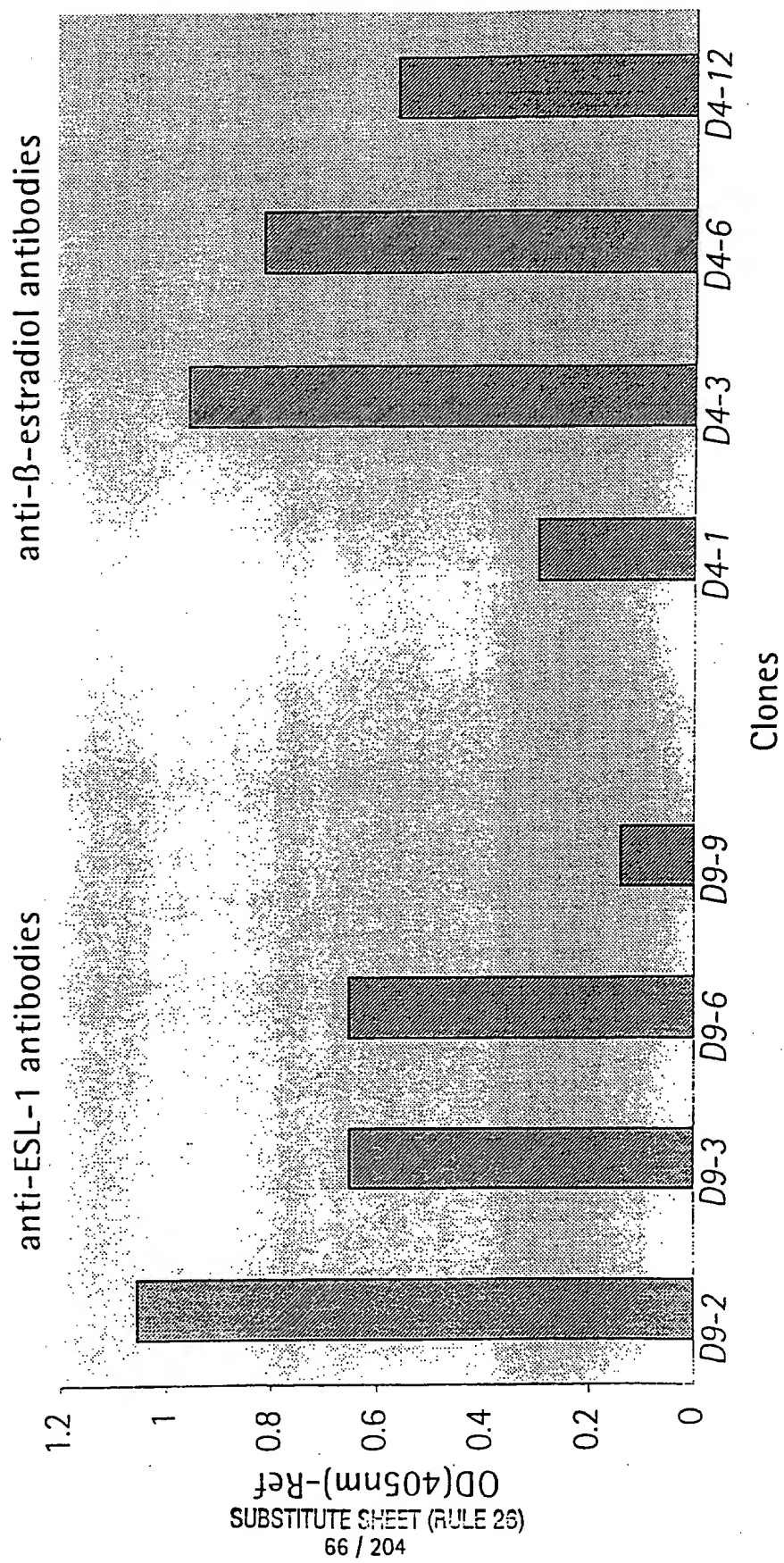
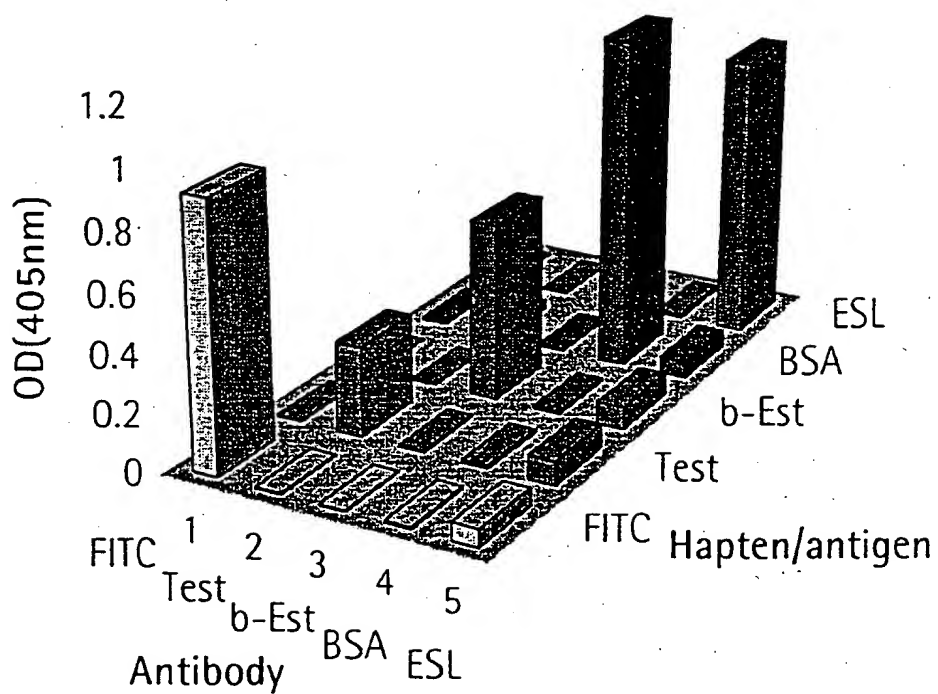


Figure 19: Selectivity and cross-reactivity of HuCAL antibodies







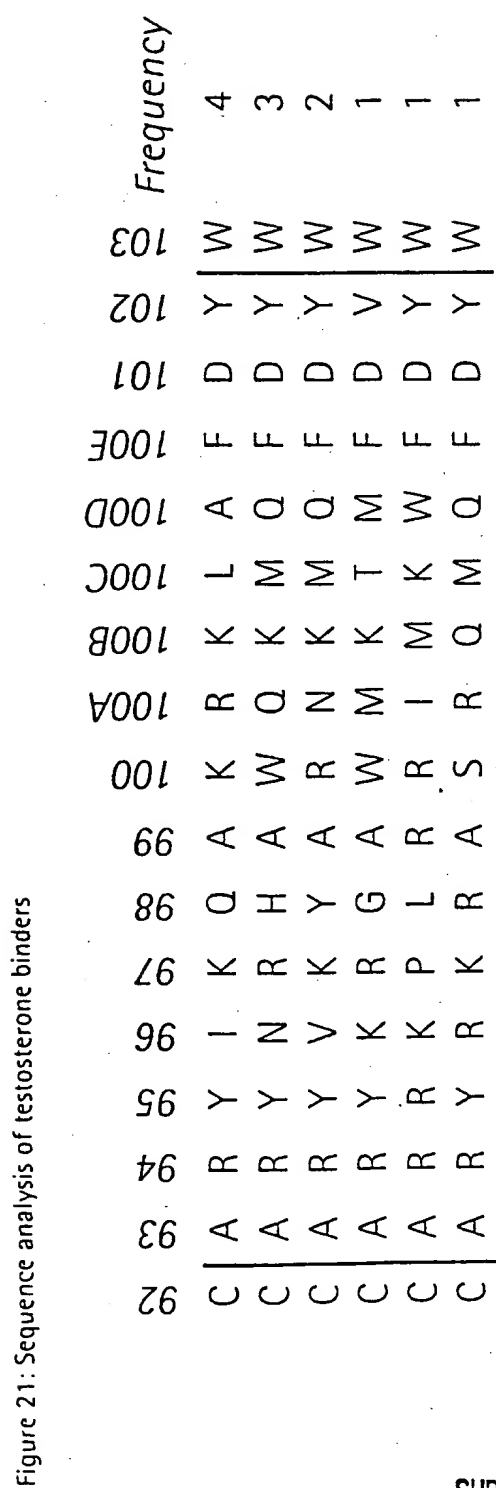


Figure 22: Sequence analysis of lymphotoxin- $\beta$  binders

| Frequency | 103 | 102 | 101 | 100F | 100D | 100C | 100B | 100A | 100 | 99 | 98 | 97 | 96 | 95 | 94 | 93 | 92 |
|-----------|-----|-----|-----|------|------|------|------|------|-----|----|----|----|----|----|----|----|----|
| 16        | W   | V   | D   | F    | H    | G    | K    | I    | K   | S  | R  | Y  | R  | Q  | R  | A  | C  |
| 1         | W   | Y   | D   | M    | P    | D    | Y    | S    | N   | F  | D  | R  | W  | M  | R  | A  | C  |
| 1         | W   | Y   | D   | F    | Q    | V    | W    | Y    | N   | D  | L  | D  | A  | L  | R  | A  | C  |
| 1         | W   | Y   | D   | M    | W    | H    | H    | P    | K   | L  | Y  | A  | Q  | L  | R  | A  | C  |
| 1         | W   | Y   | D   | M    | V    | H    | D    | R    | A   | Q  | E  | I  | W  | R  | R  | A  | C  |
| 1         | W   | V   | D   | F    | S    | Q    | T    | F    | Q   | S  | N  | H  | D  | S  | R  | A  | C  |
| 1         | W   | Y   | D   | M    | W    | E    | N    | E    | T   | Q  | F  | H  | D  | V  | R  | A  | C  |
| 1         | W   | Y   | D   | F    | W    | Y    | W    | F    | I   | L  | T  | P  | W  | D  | R  | A  | C  |

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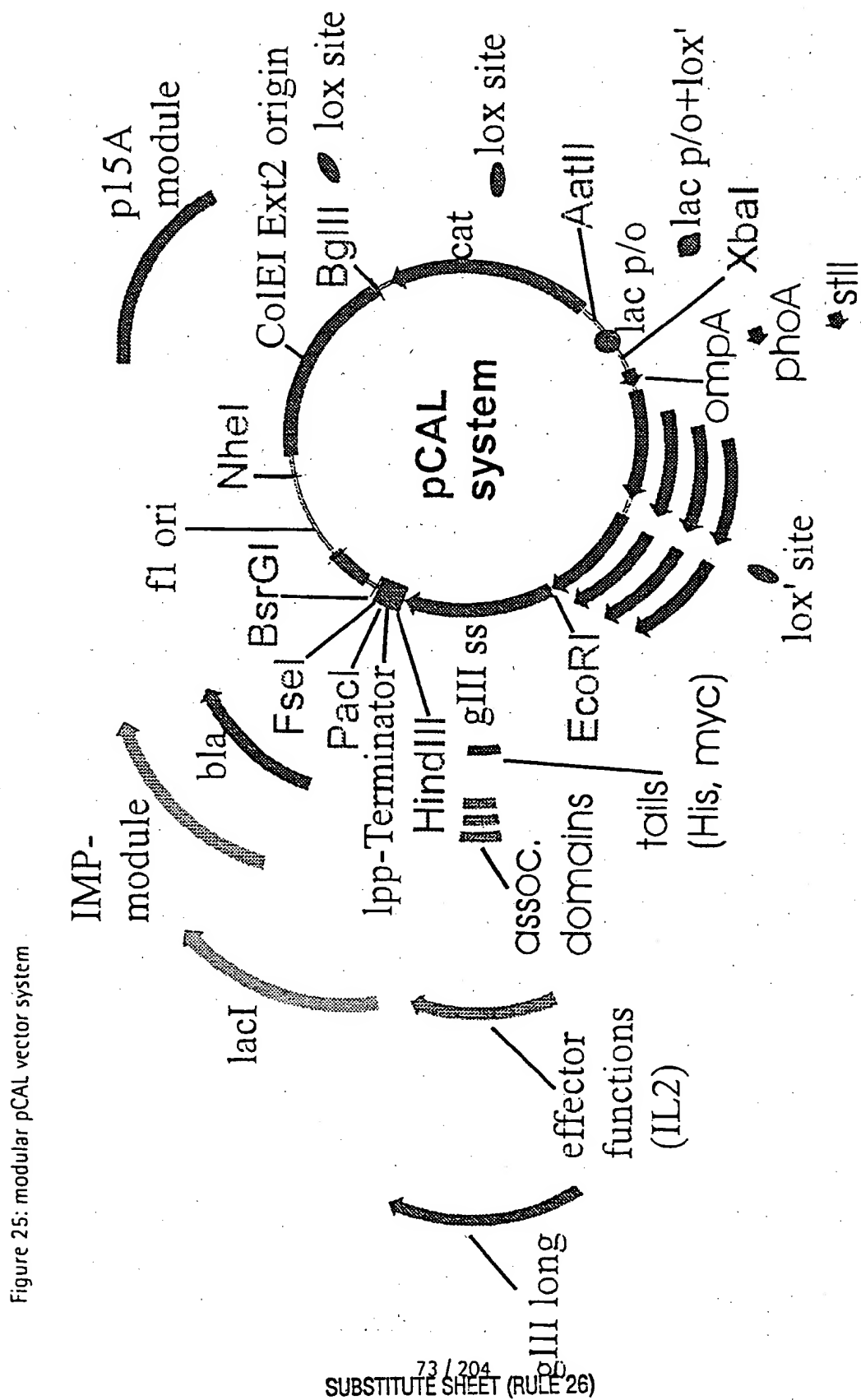


Figure 25a: List of unique restriction sites used in or suitable for HuCAL genes or pCAL vectors

| unique restriction site | Isoschizomers                     |
|-------------------------|-----------------------------------|
| AatII                   | /                                 |
| AfIII                   | BfrI, BspTI, Bst98I               |
| AscI                    | /                                 |
| Asel                    | Vspl, AsnI, PshBI                 |
| BamHI                   | BstI                              |
| BbeI                    | EheI, KasI, NarI                  |
| BbsI                    | BpuAI, BpiI                       |
| BglII                   | /                                 |
| BlpI                    | Bpu1102I, CelII, BlpI             |
| BsaBI                   | MamI, Bsh1365I, BsrBRI            |
| BsiWI                   | Pfl23II, SphI, SunI               |
| BspEI                   | AccIII, BseAI, BsiMI, Kpn2I, MroI |
| BsrGI                   | Bsp1407I, SspBI                   |
| BssHII                  | Paul                              |
| BstEII                  | BstPI, Eco91I, EcoO65I            |
| BstXI                   | /                                 |
| Bsu36I                  | AocI, CvnI, Eco81I                |
| Drall                   | /                                 |
| DsmAI                   |                                   |
| EagI                    | BstZI, EclXI, Eco52I, XmaIII      |
| Eco57I                  | /                                 |
| EcoO109I                | Drall                             |
| EcoRI                   | /                                 |
| EcoRV                   | Eco32I                            |
| FseI                    | /                                 |
| HindIII                 | /                                 |
| HpaI                    | /                                 |
| KpnI                    | Acc65I, Asp718I                   |
| MluI                    | /                                 |
| MscI                    | BalI, MluNI                       |

Figure 25a: List of unique restriction sites used in or suitable for HuCAL genes or pCAL vectors

| unique restriction site | Isoschizomers                      |
|-------------------------|------------------------------------|
| MunI                    | MfeI                               |
| NheI                    | /                                  |
| NsiI                    | Ppu10I, EcoT22I, Mph1103I          |
| NspV                    | Bsp119I, BstBI, Csp45I, LspI, SfuI |
| PacI                    | /                                  |
| PmeI                    | /                                  |
| PmlI                    | BbrPI, Eco72I, PmaCI               |
| Psp5II                  | PpuMI                              |
| PstI                    | /                                  |
| RsrII                   | (RsrI), CpoI, CspI                 |
| SanDI                   | /                                  |
| SapI                    | /                                  |
| SexAI                   | /                                  |
| SpeI                    | /                                  |
| SfiI                    | /                                  |
| SphI                    | BbuI, PaeI, NspI                   |
| StuI                    | AatI, Eco147I                      |
| StyI                    | Eco130I, EcoT14I                   |
| XbaI                    | BspLU11II                          |
| XhoI                    | PaeR7I                             |
| XmaI                    | AvaI, SmaI, Cfr9I, PspAI           |



Figure 26: list of pCAL vector modules

| No   | module/flanking restriction sites | functional element                                             | sites to be removed | sites to be inserted | template      | reference                                                                                                                           |
|------|-----------------------------------|----------------------------------------------------------------|---------------------|----------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------|
| M1   | AatII-lacp/o-XbaI                 | lac promoter/operator                                          | 2x VspI (AseI)      | AatII                | vector pASK30 | Skerra et al. (1991) Bio/Technology 9, 273-278                                                                                      |
| M2   | BglII-lox-AatII                   | Cre/lox recombination site                                     | 2x VspI (AseI)      | lox, BglII           | (synthetic)   | Hoess et al. (1986) Nucleic Acids Res. 2287-2300                                                                                    |
| M3   | XbaI-lox'-SphI                    | Cre/lox' recombination site                                    | none                | lox', SphI           | (synthetic)   | see M2                                                                                                                              |
| M7-I | EcoRI-glllong-HindIII             | glllp of filamentous phage with N-terminal myctail/amber codon | SphI, BamHI         | none                 | vector pIG10  | Ge et al., (1994) Expressing antibodies in E. coli. In: Antibody engineering: A practical approach. IRL Press, New York, pp 229-266 |

Figure 26: list of pCAL vector modules

|         |                      |                                                                          |                            |                   |              |          |
|---------|----------------------|--------------------------------------------------------------------------|----------------------------|-------------------|--------------|----------|
| M7-II   | EcoRI-gIIIss-HindIII | truncated gIIIp of filamentous phage with N-terminal Gly-Ser linker      | SphI                       |                   | vector pIG10 | see M7-I |
| M7-III  | EcoRI-gIIIss-HindIII | truncated gIIIp of filamentous phage with N-terminal myctail/amber codon | SphI, BbsI                 |                   | vector pIG10 | see M7-I |
| M8      | SphI-lox-HindIII     | Cre/lox recombination site                                               | none                       | lox               | (synthetic)  | see M3   |
| M9-II   | HindIII-lpp-PacI     | lpp-terminator                                                           | none                       | PacI, FseI        | (synthetic)  | see M1   |
| M10-II  | PacI/FseI-bla-BsrGI  | beta-lactamase/bla (ampR)                                                | VspI, Eco57I, BssSI        | PacI, FseI, BsrGI | pASK30       | see M1   |
| M11-II  | BsrGI-f1 ori-NheI    | origin of single-stranded replication                                    | DraIII (BanII not removed) | BsrGI, NheI       | pASK30       | see M1   |
| M11-III | BsrGI-f1 ori-NheI    | origin of single-stranded replication                                    | DraIII, BanII              | BsrGI, NheI       | pASK30       | see M1   |

Figure 26: list of pCAL vector modules

|          |                      |                                                       |                            |                  |             |                                                                  |
|----------|----------------------|-------------------------------------------------------|----------------------------|------------------|-------------|------------------------------------------------------------------|
| M12      | NheI-p15A-BgIII      | origin of double-stranded replication                 | BssSI, VspI, NspV          | NheI, BgIII      | pACYC184    | Rose, R.E. (1988) Nucleic Acids Res. 16, 355                     |
| M13      | BgIII-lox-BgIII      | Cre/lox recombination site                            | none                       | BgIII, lox, XmnI | (synthetic) | see M3                                                           |
| M14-Ext2 | BgIII-ColEI-NheI     | origin of double-stranded replication                 | Eco57I (BssSI not removed) | BgIII, NheI      | pUC19       | Yanisch-Peron, C. (1985) Gene 33,103-119                         |
| M17      | AatII-cat-BgIII      | chloramphenicol-acetyltransferase/cat (camR)          | BspEI, MscI, StyI/NcoI     |                  | pACYC184    | Cardoso, M. & Schwarz, S. (1992) J. Appl. Bacteriol. 72, 289-293 |
| M19      | XbaI-phoA-EcoRI      | signal sequence of phosphatase A                      | (synthetic)                |                  | (synthetic) | see M1                                                           |
| M20      | XbaI-phoA-FLAG-EcoRI | signal sequence of phosphatase A + FLAG detection tag | (synthetic)                |                  | (synthetic) | Knappik, A & Plückthun, A. (1994) BioTechniques 17, 754-761      |

Figure 26: list of pCAL vector modules

|     |                       |                                            |                                                    |  |             |                                                                               |
|-----|-----------------------|--------------------------------------------|----------------------------------------------------|--|-------------|-------------------------------------------------------------------------------|
| M21 | XbaI-stII-SapI        | heat-stable enterotoxin II signal sequence | (synthetic)                                        |  | (synthetic) | Lee et al. (1983) Infect. Immunol. 264-268                                    |
| M41 | AflII-lacI-NheI       | lac-repressor                              | BstXI, MluI, BbsI, BanII, BstEII, HpaI, BbeI, VspI |  | pASK30      | see M1                                                                        |
| M42 | EcoRI-Histail-HindIII | poly-histidine tail                        | (synthetic)                                        |  | (synthetic) | Lindner et al., (1992) Methods: a companion to methods in enzymology 4, 41-56 |

Figure 27: functional map and sequence of MCS module

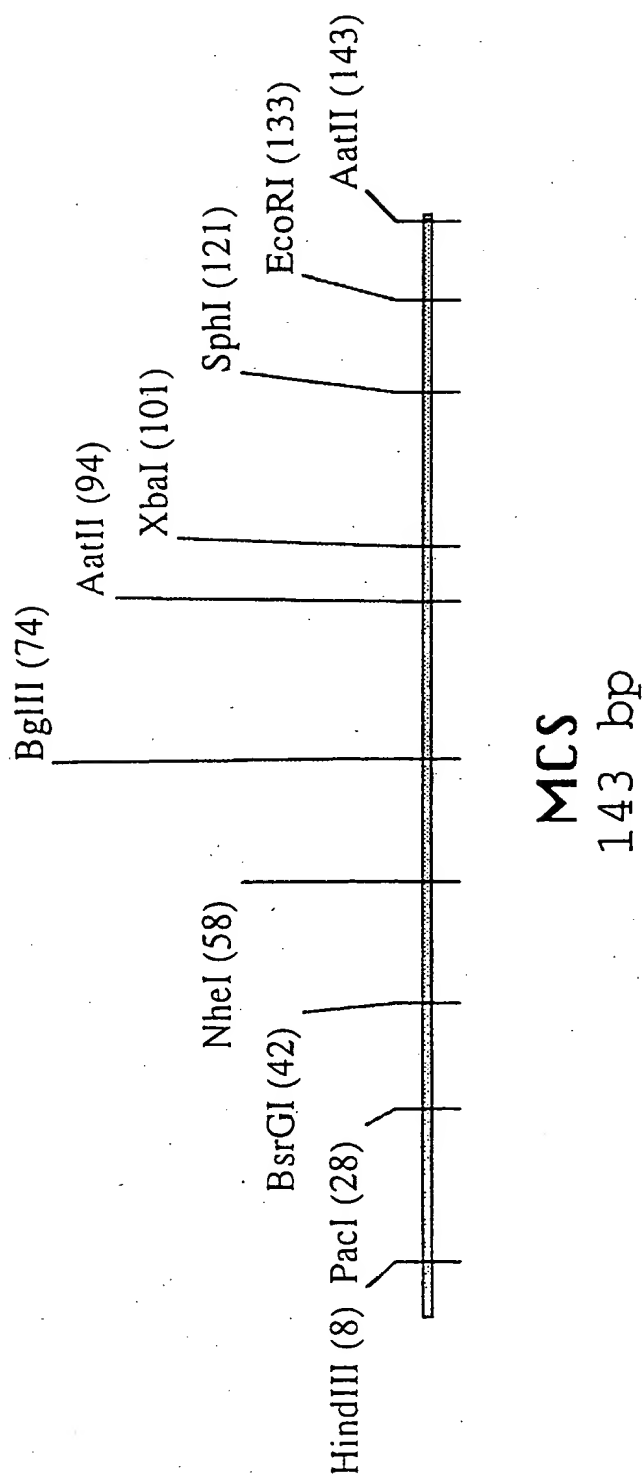


Figure 27: functional map and sequence of MCS module (continued)

|     |                                                        |       |             |
|-----|--------------------------------------------------------|-------|-------------|
|     | HindIII                                                | PacI  | BsrGI       |
|     | ~~~~~                                                  | ~~~~~ | ~~~~~       |
| 1   | ACATGTAAGC TTCCCCCCCC CCTTAATTAA CCCCCCCCC TGTACACCCC  |       |             |
|     | TGTACATTTCG AAGGGGGGGG GGAATTAATT GGGGGGGGG ACATGTGGGG |       |             |
|     | NheI                                                   | BglII | AatII XbaI  |
|     | ~~~~~                                                  | ~~~~~ | ~~~~~       |
| 51  | CCCCCGGCTA GCCCCCCCCC CCAGATCTCC CCCCCCCCC GTCCCCCCT   |       |             |
|     | GGGGGGCGAT CGGGGGGGGG GGTCTAGAGG GGGGGGGCT GCAGGGGGGA  |       |             |
|     | XbaI                                                   | SphI  | EcoRI AatII |
|     | ~~~~~                                                  | ~~~~~ | ~~~~~       |
| 101 | CTAGACCCCC CCCCCGCATG CCCCCCCCC CGAATTCGAC GTC         |       |             |
|     | GATCTGGGG GGGGGCGTAC GGGGGGGGG GCTTAAGCTG CAG          |       |             |

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Figure 28: functional map and sequence of pMCS cloning vector

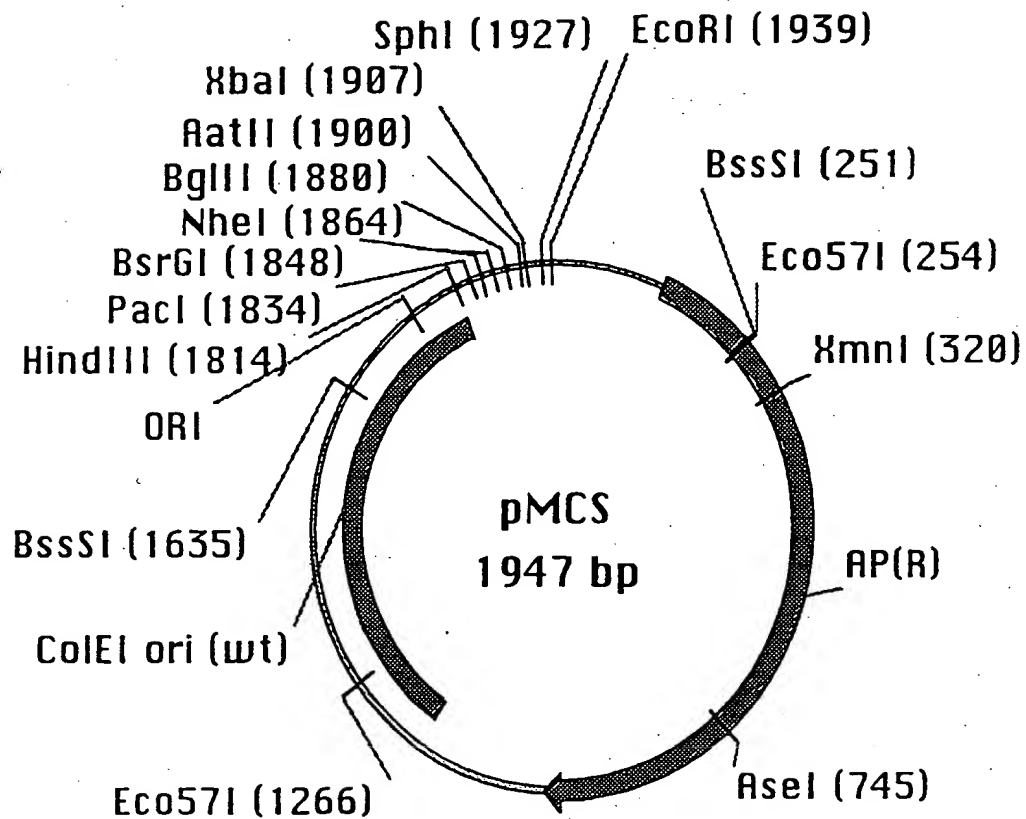


Figure 28: functional map and sequence of pMCS cloning vector (continued)

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1  CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATT
   GTCCACCGTG AAAAGCCCTT TTACACGCGC CTGGGGGATA AACAAATAAA

51 TTCTAAATAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCCTGATA
   AAGATTATG TAAGTTTATA CATAGGCGAG TACTCTGTTA TTGGGACTAT

101 AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC
   TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTGTAAAGG

151 GTGTCGCCCT TATTCCTTTT TTGCGGCAT TTGCCCTTCC TGTTTTGGCT
   CACAGCGGGA ATAAGGGAAA AAACGCCGTA AACGGAAGG ACAAAAACGA

                                     Eco57I
                                     ~~~~~

201 CACCCAGAAA CGCTGGTGAA AGTAAAGAT GCTGAAGATC AGTTGGGTGC
   GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG
                                     BssSI
                                     ~~~~~

251 ACGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA
   TGCTCACCCA ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACTCT
   BssSI
   ~~~~~

```



Figure 28: functional map and sequence of pMCS cloning vector (continued)

## Xmn I

```

301 GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG
    CAAAGCGGG GCTTCTTGCA AAAGTTACT ACTCGTGAAA ATTTCAAGAC

351 CTATGTGGCG CGGTATTATC CCGTATTGAC GCCGGGCAAG AGCAACTCGG
    GATACACCGC GCCATAATAG GGCATAACTG CGGCCCCGTT CCGTTGAGCC

401 TCGCCGCATA CACTATTCTC AGAATGACTT GGTGAGTAC TCACCAGTCA
    AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT

451 CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAAAT ATGCAGTGCT
    GTCTTTTCGT AGAATGCCTA CCGTACTGTC ATTCTCTTAA TACGTCACGA

501 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT
    CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA

551 CGGAGGACCG AAGGAGCTAA CCGCTTTTTC GCACAACATG GGGGATCATG
    GCCTCCTGGC TTCCTCGATT GCGAAAAAAA CGTGTGTAC CCCCTAGTAC

601 TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC
    ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTACTTCG GTATGGTTTG

651 GACGAGCGTG ACACCACGAT GCCTGTAGCA ATGGCAACAA CGTTGCGCAA

```

Figure 28: functional map and sequence of pMCS cloning vector (continued)

|      |            |            |            |             |            |
|------|------------|------------|------------|-------------|------------|
|      | CTGCTCGCAC | TGTGGTGCTA | CGGACATCGT | TACCGTTGTT  | GCAACGCGTT |
|      |            |            |            |             | AseI       |
|      |            |            |            |             | ~~~~~      |
| 701  | ACTATTAACT | GGCGAACTAC | TTACTCTAGC | TTCCCGGCAA  | CAATTAATAG |
|      | TGATAATTGA | CCGCTTGATG | AATGAGATCG | AAGGCCGTT   | GTTAATTATC |
| 751  | ACTGGATGGA | GGCGGATAAA | GTTGCAGGAC | CACCTCTGCG  | CTCGGCCCTT |
|      | TGACCTACCT | CCGCCATT   | CAACGTCC   | GTGAAGACGC  | GAGCCGGGAA |
| 801  | CCGGCTGGCT | GGTTATTGC  | TGATAAATCT | GGAGCCGGTG  | AGCGTGGGTC |
|      | GGCCGACCGA | CCAAATAACG | ACTATTTAGA | CCTCGGCCAC  | TCGCACCCAG |
| 851  | TCGCGGTATC | ATTGCAGCAC | TGGGGCCAGA | TGGTAAGCCC  | TCCCGTATCG |
|      | AGCGCCATAG | TAACGTCGTG | ACCCCGGTCT | ACCATTCGGG  | AGGCGATAGC |
| 901  | TAGTTATCTA | CACGACGGGG | AGTCAGGCAA | CTATGGATGA  | ACGAAATAGA |
|      | ATCAATAGAT | GTGCTGCCCC | TCAGTCCGTT | GATACCTACT  | TGCTTTATCT |
| 951  | CAGATCGCTG | AGATAGGTGC | CTCACTGATT | AAGCATTTGGT | AACGTGCAGA |
|      | GTCTAGCGAC | TCTATCCACG | GAGTGACTAA | TTCGTAACCA  | TTGACAGTCT |
| 1001 | CCAAGTTTAC | TCATATATAC | TTTAGATTGA | TTTAAAACCTT | CATTTTAAAT |
|      | GGTTCAAATG | AGTATATATG | AAATCTAACT | AAATTTTGAA  | GTAAAAATTA |

Figure 28: functional map and sequence of pMCS cloning vector (continued)

```

1051  TTAAGGAT CTAGGTGAAG ATCCTTTTGT ATAATCTCAT GACCAAAATC
      AATTTTCCTA GATCCACTTC TAGGAAAAC TATTAGAGTA CTGGTTTTAG

1101  CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT
      GGAATTGCAC TCAAAAGCAA GTGACTCGC AGTCTGGGC ATCTTTTCTA

1151  CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC
      GTTTCCTAGA AGAACTCTAG GAAAAAAGA CGCGCATTAG ACGACGAACG

1201  AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG
      TTTGTTTTTT TGGTGGCGAT GTCGCCACC AAACAAACGG CCTAGTTCTC

1251  CTACCAACTC TTTTCCGAA GGTAAGTGGC TTCAGCAGAG CGCAGATACC
      GATGGTTGAG AAAAAGGCTT CCATTGACCG AAGTCGTCTC GCGTCTATGG
                                     Eco57I
                                     ~~~~~

1301  AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT
      TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA

1351  CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT
      GACATCGTGG CGGATGTATG GAGCGAGACG ATTAGGACAA TGGTCACCCGA

```

Figure 28: functional map and sequence of pMCS cloning vector (continued)

|      |             |             |             |             |            |
|------|-------------|-------------|-------------|-------------|------------|
| 1401 | GCTGCCAGTG  | CGGATAAGTC  | GTGTCTTACC  | GGGTTGGACT  | CAAGACGATA |
|      | CGACGGTCAC  | CGCTATTTCAG | CACAGAAATGG | CCCAACCTGA  | GTCTGTCTAT |
| 1451 | GTTACCGGAT  | AAGGCGCAGC  | GGTCGGGCTG  | AACGGGGGGT  | TCGTGCACAC |
|      | CAATGGCCCTA | TTCCGCCGTCG | CCAGCCCCGAC | TTGCCCCCCCA | AGCACGTGTG |
| 1501 | AGCCCAGCTT  | GGAGCGAACG  | ACCTACACCG  | AAC TGAGATA | CCTACAGCGT |
|      | TCGGGTCGAA  | CCTCGCTTGC  | TGGATGTGGC  | TTGACTCTAT  | GGATGTCCGA |
| 1551 | GAGCTATGAG  | AAAGCGCCAC  | GCTTCCCAGAA | GGGAGAAAGG  | CGGACAGGTA |
|      | CTCGATACTC  | TTTCGCCGGTG | CGAAGGGCTT  | CCCTCTTTCC  | GCCTGTCCAT |
| 1601 | TCCGGTAAGC  | GGCAGGGTCG  | GAACAGGAGA  | GCGCACGAGG  | GAGCTTCCAG |
|      | AGGCCATTTCG | CCGTCCCAGC  | CTTGTCCTCT  | CGCGTGCTCC  | CTCGAAGGTC |
|      |             |             |             | BSSI        |            |
|      |             |             |             | ~~~~~       |            |
| 1651 | GGGGAACGC   | CTGGTATCTT  | TATAGTCCTG  | TCGGGTTTTCG | CCACCTCTGA |
|      | CCCCTTTGCG  | GACCATAGAA  | ATATCAGGAC  | AGCCCCAAGC  | GGTGGAGACT |
| 1701 | CTTGAGCGTC  | GATTTTGTG   | ATGCTCGTCA  | GGGGGGCGGA  | GCCTATGGAA |
|      | GAACTCGCAG  | CTAAAAACAC  | TACGAGCAGT  | CCCCCCGCCT  | CGGATACCTT |
| 1751 | AAACGCCAGC  | AACGCGGCCT  | TTTTACGGTT  | CCTGGCCCTT  | TGCTGGCCTT |

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Figure 28: functional map and sequence of pMCS cloning vector (continued)

```

TTTGCGGTCG TTGCGCCGGA AAAATGCCAA GGACCGGAAA ACGACCGGAA

      HindIII              PacI              BsrGI
      ~~~~~              ~~~~~              ~~~~~
1801 TTGCTCACAT GTAAGCTTCC CCCCCCCTT AATAACCCC CCCCCCTGTA
AACGAGTGTA CATTCAAGG GGGGGGGA TTAATTGGG GGGGGACAT

      BsrGI              NheI              BglII              AatII
      ~~~              ~~~~~              ~~~~~              ~~~~~
1851 CACCCCCCCC CCGTAGCCC CCCCCCCC ATCTCCCCC CCCCACGTC
GTGGGGGGG GCGATCGG GGGGGGGG TAGAGGGGG GGGCTGCAG

      XbaI              SphI              EcoRI
      ~~~~~              ~~~~~              ~~~~~
1901 CCCCCTCTAG ACCCCCCCCC CGCATGCCCC CCCCCCGAA TTCACGT
GGGGGAGATC TGGGGGGGG GCGTACGGG GGGGGGCTT AAGTGCA

```

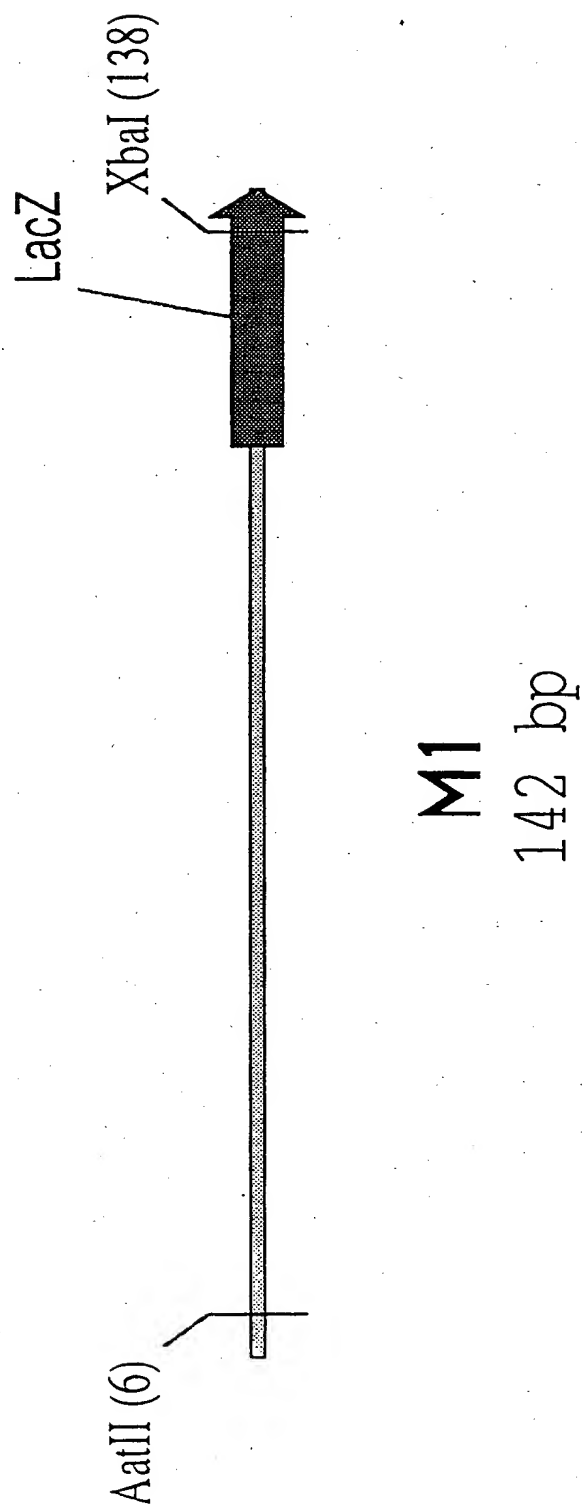


Figure 29: functional map and sequence of pCAL module M1

Figure 29: functional map and sequence of pCAL module M1

AatII  
~~~~~

1 GACGTCCTTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA GGCTTTACAC  
CTGCAGAAAT ACACCTCAATC GAGTGAGTAA TCCGTGGGGT CCGAAATGTG

51 TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT  
AAATACGAAG GCCGAGCATA CAACACACCT TAACACTCGC CTATTGTTAA

XbaI  
~~~~~

101 TCACACAGGA AACAGCTATG ACCATGATTA CGAATTCTA GA  
AGTGTGTCCT TTGTCGATAC TGGTACTAAT GCTTAAAGAT CT

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Figure 30: functional map and sequence of pCAL module M7-II

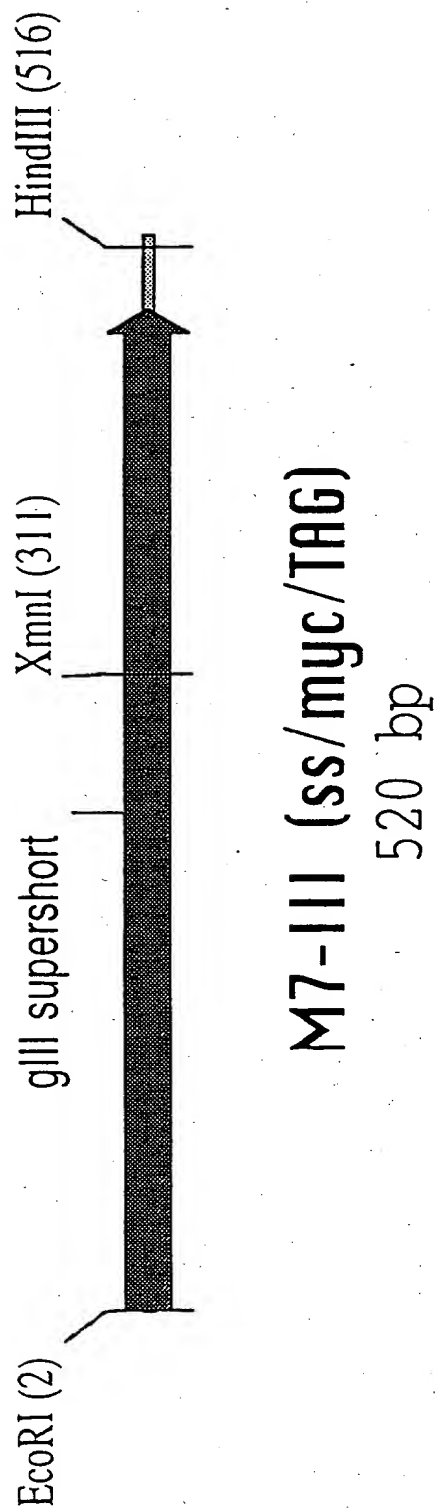




Figure 30: functional map and sequence of pCAL module M7-II (continued)

EcoRI  
 ~~~~~  
 1 GAATTCGAGC AGAAGCTGAT CTCTGAGGAG GATCTGTAGG GTGGTGGCTC  
 CTTAAGCTCG TCTTCGACTA GAGACTCCTC CTAGACATCC CACCACCGAG  
 51 TGGTTCGGGT GATTTTGATT ATGAAAAGAT GGCAACGCT AATAAGGGG  
 ACCAAGGCCA CTAAAACTAA TACTTTTCTA CCGTTTGCGA TTATTCCCCC  
 101 CTATGACCGA AAATGCCGAT GAAACGCGC TACAGTCTGA CGCTAAAGGC  
 GATACTGGCT TTTACGGCTA CTTTGGCGG ATGTCAGACT GCGATTTCGG  
 151 AAACCTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT  
 TTTGAACATA GACAGCGATG ACTAATGCCA CGACGATAGC TACCAAAGTA  
 201 TGGTGACGTT TCCGGCCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTG  
 ACCACTGCAA AGCCCGGAAC GATTACCAT ACCACGATGA CCACTAAAC  
 251 CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT  
 GACCGAGATT AAGGGTTTAC CGAGTTCAGC CACTGCCACT ATTAAGTGGA  
 XmnI  
 ~~~~~  
 301 TTAATGAATA ATTTCCGTCA ATATTACCT TCCCTCCCTC AATCGGTGA  
 AATTACTTAT TAAAGGCAGT TATAAATGGA AGGAGGGAG TTAGCCAACT

Figure 30: functional map and sequence of pCAL module M7-II (continued)

```
351  ATGTCGCCCT  TTTGTCTTTG  GCGCTGGTAA  ACCATATGAA  TTTTCTATTG
    TACAGCGGGA  AACAGAAAC  CGGACCAT  TGGTATACTT  AAAAGATAAC

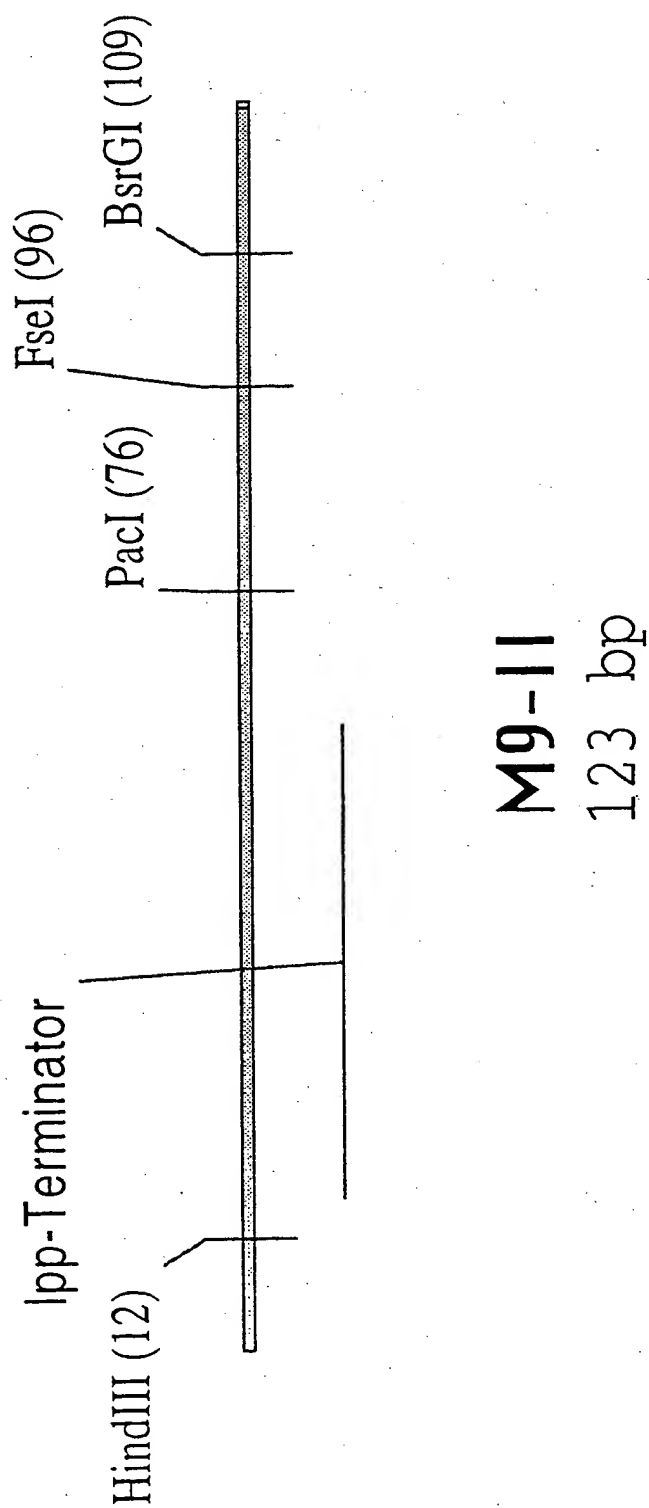
401  ATTGTGACAA  AATAAACTTA  TTCCGTGGTG  TCCTTGCGTT  TCTTTTATAT
    TAACACTGTT  TTATTTGAAT  AAGCACCCAC  AGAAACGCAA  AGAAAATATA

451  GTTGCCACCT  TTATGTATGT  ATTTCTACG  TTTGCTAACA  TACTGCGTAA
    CAACGGTGA  AATACATACA  TAAAGATGC  AAACGATTGT  ATGACGCATT

                    HindIII
                    ~~~~~

501  TAAGGAGTCT  TGATAAGCTT
    ATTCCTCAGA  ACTATTTCGAA
```

Figure 31: functional map and sequence of pCAL module M9-II



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Figure 31: functional map and sequence of pCAL module M9-II (continued)

```

HindIII
~~~~~
1  GGGGGGGGGG AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG
   CCCCCCCCCC TTCGAACTGG ACACTTCACT TTTTACCGCG TCTAACACGC

PacI
~~~~~
51 ACATTTT TGTCTGCCGT TTAATTAAAG GGGGGGGGGG GCCGGCCTGG
   TGTAATAAAA ACAGACGGCA AATTAATTTC CCCCCCCCCC CGCCGGGACC

FseI
~~~~~

BsrGI
~~~~~
101 GGGGGGGTGT ACAGGGGGGG GGG
    CCCCCCCACA TGTCCCCCCC CCC

```

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Figure 32: functional map and sequence of pCAL module M11-III

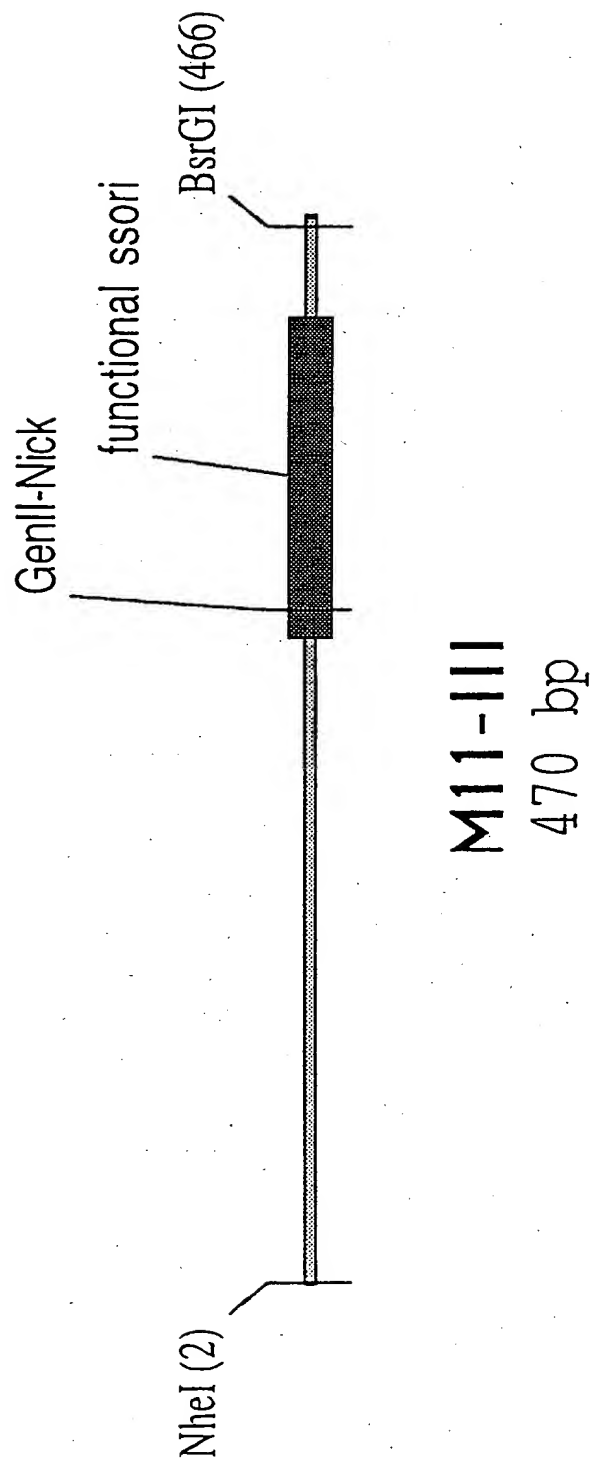


Figure 32: functional map and sequence of pCAL module M11-III (continued)

NheI  
~~~~~

1	GCTAGCACGC	GCCCTGTAGC	GGCGCATTA	GGCGGCGGG	TGTGTTGGTT
	CGATCGTGCG	CGGACATCG	CCGCGTAATT	CGCGCCGCC	ACACCACCAA
51	ACGCGCAGCG	TGACCGCTAC	ACTTGCCAGC	GCCCTAGCGC	CCGCTCCTTT
	TGCGCGTCGC	ACTGGCGATG	TGAACGGTCG	CGGATCGCG	GGCGAGGAAA
101	CGCTTTCTTC	CCTTCCCTTC	TCGCCACGTT	CGCCGGCTTT	CCCCGTCAAG
	GCGAAAGAAG	GGAAGGAAAG	AGCGGTGCAA	GCGGCCGAAA	GGGCAGTTC
151	CTCTAAATCG	GGGCATCCCT	TTAGGGTTCC	GATTAGTGC	TTACGGCAC
	GAGATTTAGC	CCCGTAGGGA	AATCCCAAGG	CTAAATCACG	AAATGCCGTG
201	CTCGACCCCA	AAAAACTTGA	TTAGGGTGAT	GGTTCTCGTA	GTGGGCCATC
	GAGCTGGGGT	TTTTTGAACT	AATCCCACTA	CCAAGAGCAT	CACCCGGTAG
251	GCCCTGATAG	ACGGTTTTC	GCCCTTTGAC	GTTGGAGTCC	ACGTTCTTTA
	CGGGAATATC	TGCCAAAAG	CGGAAACTG	CAACCTCAGG	TGCAAGAAAT
301	ATAGTGGAAT	CTTGTTCCTA	ACTGGAACAA	CACTCAACCC	TATCTCGGTC
	TATCACCTGA	GAACAAGGTT	TGACCTTGT	GTGAGTTGGG	ATAGAGCCAG
351	TATTCTTTTG	ATTATAAGG	GATTTGCCG	ATTTCGGCCT	ATTGGTTAAA

Figure 32: functional map and sequence of pCAL module M11-III (continued)

ATAAGAAAAC TAAATATTCC CTAAAACGGC TAAAGCCGGA TAACCAATTT

401 AAATGAGCTG ATTTAACAAA AATTTAACGC GAATTTTAAAC AAAATATTA  
TTTACTCGAC TAAATTGTTT TTAAATTGCG CTTAAAAATTG TTTTATAATT

BsrGI

~~~~~

451 CGTTACAAT TTCATGTACA  
GCAATGTTA AAGTACATGT

Figure 33: functional map and sequence of pCAL module M14-Ext2

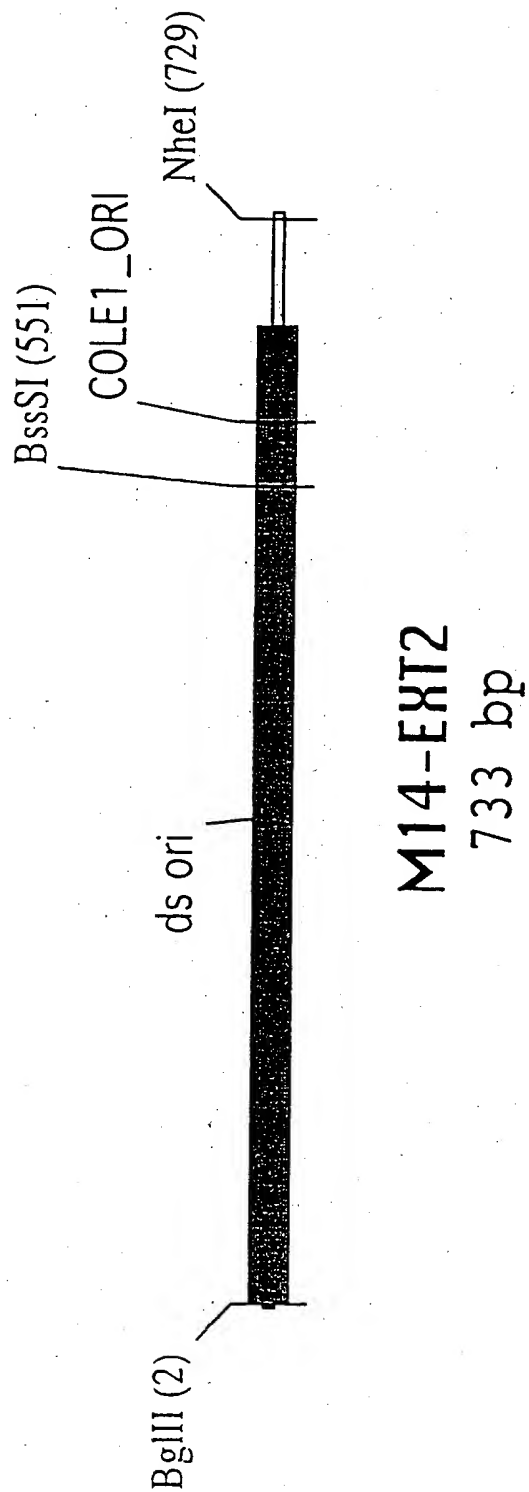




Figure 33: functional map and sequence of pCAL module M14-Ext2 (continued)

## Bg1 II

```

1  AGATCTGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG
   TCTAGACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC

51  ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCCTT TTTTCTGCGC
   TGGGCGATCT TTTCTAGTTT CCTAGAAGAA CTCTAGGAAA AAAAGACGCG

101 GTAATCTGCT GCTTGCAAAC AAAAAACCA CCGCTACCAG CCGTGGTTTG
   CATTAGACGA CGAACGTTTG TTTTTTTGGT GCGGATGGTC GCCACCAAAC

151 TTTGCCGGAT CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTACA
   AAACGGCCCTA GTTCTCGATG GTTGAGAAAA AGGCTTCCAT TGACCGATGT

201 GCAGAGCGCA GATACCAAAT ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC
   CGTCTCGCGT CTATGGTTTA TGACAAGAAG ATCACATCGG CATCAATCCG

251 CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG CTCTGCTAAT
   GTGGTGAAGT TCTTGAGACA TCGTGGCGGA TGTATGGAGC GAGACGATTA

301 CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT
   GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCCA

351 TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG

```

Figure 33: functional map and sequence of pCAL module M14-Ext2 (continued)

```

ACCTGAGTTC TGCTATCAAT GGCCATTATCC GCGTCGCCAG CCCGACTTGC

401 GGGGGTTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT
CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA

451 GAGATACCTA CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAAGGA
CTCTATGGAT GTCGCACTCG ATACTCTTTC GCGGTGCGAA GGGCTTCCCT

501 GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGTTCGGAAC AGGAGAGCGC
CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG
BssSI

551 ACGAGGGAGC TTCCAGGGGG AAACGCCCTGG TATCTTTATA GTCCCTGTCCG
TGCTCCCCTCG AAGTCCCCC TTTGCGGACC ATAGAAATAT CAGGACAGCC
BssSI
~~~~~

601 GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG
CAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC

651 GGCGGAGCCT ATGGA AAAAC GCCAGCAACG CGGCCCTTTT ACGGTTCCCTG
CGCCCTCGGA TACCTTTTTC GGTCTGTTGC GCCGAAAAA TGCCAAAGGAC

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Figure 33: functional map and sequence of pCAL module M14-Ext2 (continued)

701 GCCTTTTGCT GGCCTTTTGC TCACATGGCT AGC  
CGGAAAACGA CCGGAAAACG AGTGTACCGA TCG

NheI  
~~~~~

Figure 34: functional map and sequence of pCAL module M17

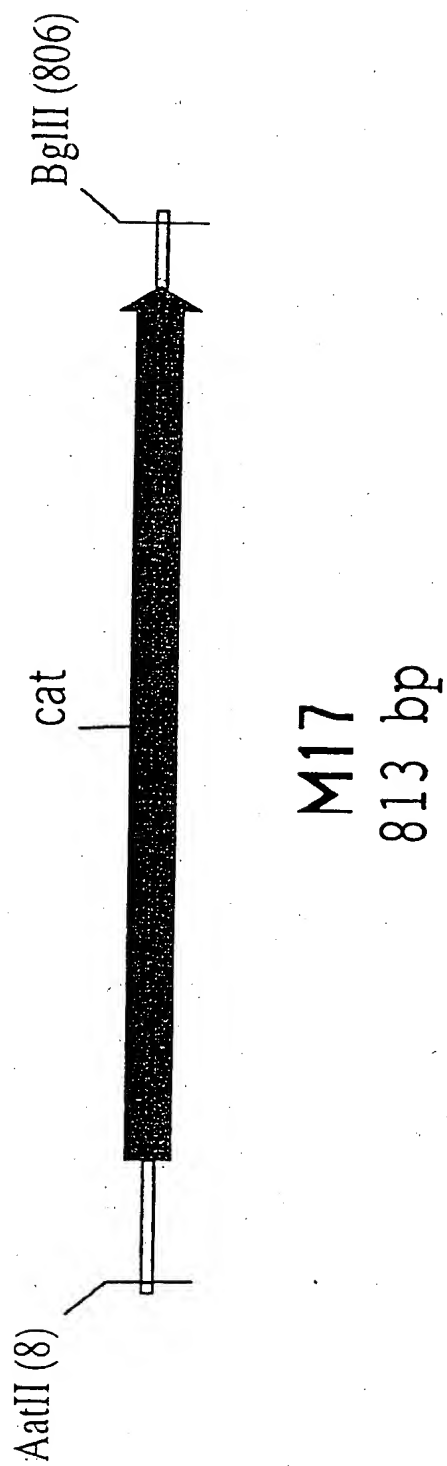


Figure 34: functional map and sequence of pCAL module M17 (continued)

## AatII

```

1  GGGACGTCGG GTGAGGTTCC AACTTTCACC ATAATGAAAT AAGATCACTA
   CCTGCAGCC CACTCCAAGG TTGAAAGTGG TATTACTTTA TTCTAGTGAT

51  CCGGGCGTAT TTTTGTGAGTT ATCGAGATTT TCAGGAGCTA AGGAAGCTAA
   GGCCCGCATA AAAAACTCAA TAGCTCTAAA AGTCCTCGAT TCCTTCGATT

101 AATGGAGAAA AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC
   TTACCTCTTT TTTTAGTGAC CTATATGGTG GCAACTATAT AGGGTTACCG

151 ATCGTAAAGA ACATTTTGAG GCATTTCAGT CAGTTGCTCA ATGTACCTAT
   TAGCATTTCT TGTAAACTC CGTAAAGTCA GTCACGAGT TACATGGATA

201 AACCAGACCG TTCAGCTGGA TATTACGGCC TTTTAAAGA CCGTAAAGAA
   TTGGTCTGGC AAGTCGACCT ATAATGCCGG AAAAATTCTT GGCATTTCTT

251 AAATAAGCAC AAGTTTATC CGGCCCTTAT TCACATTCTT GCCCGCCTGA
   TTTATTCTGT TTCAAATAG GCCGAAATA AGTGTAAAGA CGGCGGACT

301 TGAATGCTCA CCCGGAGTTC CGTATGGCAA TGAAGACGG TGAGCTGGTG
   ACTTACGAGT GGGCCTCAAG GCATACCGTT ACTTCTGCC ACTCGACCAC

351 ATATGGGATA GTGTTACCC TTGTACACC GTTTCCATG AGCAAACCTGA

```

Figure 34: functional map and sequence of pCAL module M17 (continued)

|     |             |            |            |             |             |
|-----|-------------|------------|------------|-------------|-------------|
|     | TATACCCCTAT | CACAAGTGGG | AACAATGTGG | CAAAAGGTAC  | TCGTTTGACT  |
| 401 | AACGTTTCA   | TCGCTCTGGA | GTGAATACCA | CGACGATTTC  | CGGCAGTTTC  |
|     | TTGCAAAAGT  | AGCGAGACCT | CACCTATGGT | GCTGCTAAAG  | GCCGTCAAAG  |
| 451 | TACACATATA  | TTCGCAAGAT | GTGGCGTGTT | ACGGTGAAAA  | CCTGGCCCTAT |
|     | ATGTGTATAT  | AAGCGTTCTA | CACCGCACAA | TGCCACTTTT  | GGACCGGATA  |
| 501 | TTCCCTAAAG  | GGTTTATTGA | GAATATGTTT | TTCGTCTCAG  | CCAATCCCCTG |
|     | AAGGGATTTC  | CCAAATAACT | CTTATACAAA | AAGCAGAGTC  | GTTAGGGAC   |
| 551 | GGTGAGTTTC  | ACCAGTTTGT | ATTTAAACGT | AGCCAATATG  | GACAACTTCT  |
|     | CCACTCAAAG  | TGGTCAAAAC | TAAATTGCA  | TCGGTTATAC  | CTGTTGAAGA  |
| 601 | TCGCCCCCGT  | TTTCACTATG | GGCAAATATT | ATACGCAAGG  | CGACAAGGTG  |
|     | AGCGGGGGCA  | AAAGTGATAC | CCGTTTATAA | TATGCGTTCC  | GCTGTTCCAC  |
| 651 | CTGATGCCCG  | TGGCGATTCA | GGTTCATCAT | GCCGTTTGTTG | ATGGCTTCCA  |
|     | GACTACGGCG  | ACCGCTAAGT | CCAAGTAGTA | CGGCAAAACAC | TACCGAAGGT  |
| 701 | TGTCGGCAGA  | ATGCTTAATG | AATTACAACA | GTAATGCGAT  | GAGTGGCAGG  |
|     | ACAGCCGTCT  | TACGAATTAC | TTAATGTTGT | CATGACGCTA  | CTCACCGTCC  |
| 751 | GCGGGGCGTA  | ATTTTTTAA  | GGCAGTTATT | GGGTGCCCTT  | AAACGCCCTGG |

Figure 34: functional map and sequence of pCAL-module M17 (continued)

CGCCCCGCAT TAAAAAATT CCGTCAATAA CCCACGGGAA TTGCGGACC

BglII  
~~~~~

801 TGCTAGATCT TCC  
ACGATCTAGA AGG

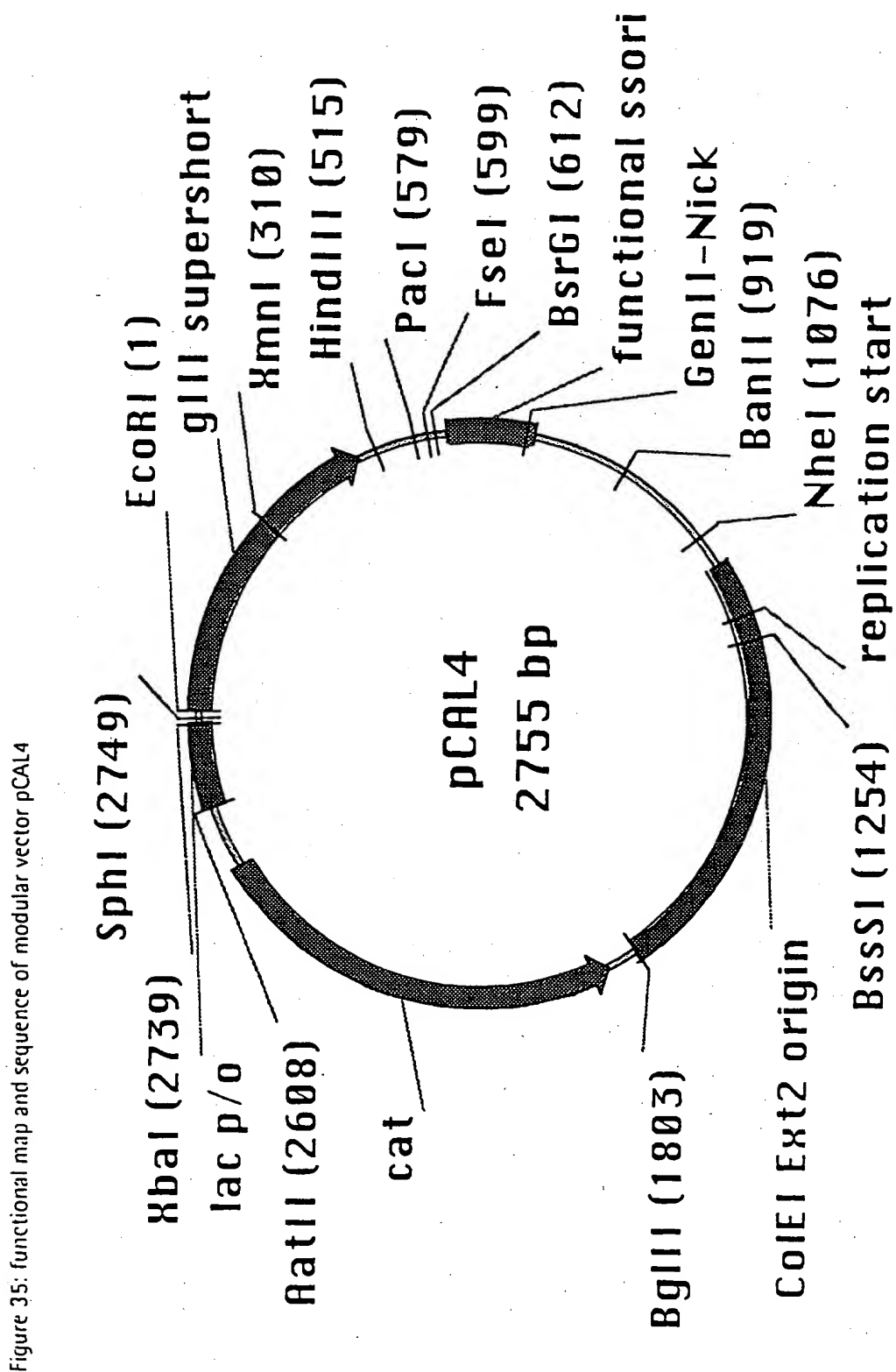




Figure 35: functional map and sequence of modular vector pCAL4 (continued)

ECORI  
 ~~~~~  
 1 AATTCGAGCA GAAGCTGATC TCTGAGGAGG ATCTGTAGGG TGGTGGCTCT  
 TTAAGCTCGT CTTGACTAG AGACTCCTCC TAGACATCCC ACCACCGAGA  
  
 51 GGTTCGGTG ATTTTGATTA TGAAAAGATG GCAAACGCTA ATAAGGGGC  
 CCAAGGCCAC TAAAACTAAT ACTTTCTAC CGTTTGGGAT TATTCCTCCG  
  
 101 TATGACCGAA AATGCCGATG AAAACGCGCT ACAGTCTGAC GCTAAAGGCA  
 ATACTGGCTT TTACGGCTAC TTTTGCGCGA TGTGAGACTG CGATTTCCGT  
  
 151 AACTTGATTC TGTGCTACT GATTACGGTG CTGCTATCGA TGGTTTCATT  
 TTGAACTAAG ACAGCGATGA CTAATGCCAC GACGATAGCT ACCAAAGTAA  
  
 201 GGTGACGTT CCGGCTTGC TAATGGTAAT GGTGCTACTG GTGATTTTGC  
 CCACTGCAA GCGCGGAACG ATTACCATTA CCACGATGAC CACTAAAACG  
  
 251 TGGCTCTAAT TCCCAAATGG CTCAAGTCGG TGACGGTGAT AATTCACCTT  
 ACCGAGATTA AGGTTTACC GAGTTCAGCC ACTGCCACTA TTAAGTGGAA  
  
 XmnI  
 ~~~~~  
 301 TAATGAATAA TTTCCGTCAA TATTACCTT CCCCTCCCTCA ATCGGTTGAA  
 ATTACTTATT AAAGGCAGTT ATAAATGGAA GGGAGGGAGT TAGCCAACTT

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

```

351  TGTCGCCCTT TTGTCTTTGG CGCTGGTAA  CCATATGAAT  TTTCTATTGA
    ACAGCGGGAA AACAGAAACC GCGACCATTT  GGTATACTTA  AAAGATAACT

401  TTGTGACAAA ATAAACTTAT TCCGTGGTGT  CTTTGCGTTT  CTTTATATATG
    AACACTGTTT TATTTGAATA AGGCACCACA  GAAACGCCAA  GAAAATATAC

451  TTGCCACCCTT TATGTATGTA TTTTCTACGT  TTGCTAACAT  ACTGCCGTAAT
    AACGGTGGAA ATACATACAT AAAAGATGCA  AACGATTGTA  TGACGCATTA

      HindIII
      ~~~~~

501  AAGGAGTCTT GATAAGCTTG ACCTGTGAAG  TGAAAAATGG  CGCAGATTGT
    TTCCTCAGAA CTATTCGAAC TGGACACTTC  ACTTTTACC  GCGTCTAACA

      PacI
      ~~~~~

551  GCGACATTTT TTTTGTCTGC CGTTTAATTA  AAGGGGGGGG  GGGCCCGGCC
    CGCTGTAAAA AAAACAGACG GCAAATTAAT  TTCCCCCCCC  CCCC GGCCGG

      BsrGI
      ~~~~~

601  TGGGGGGGGG TGTACATGAA ATTGTAAACG  TTAATATTTT  GTTAAATTC
    ACCCCCCCCC ACATGTACTT TAACATTTCG  AATTATAAAA  CAATTTTAAG

```

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

```

651  GCGTTAAATT TTTGTTAAAT CAGCTCATTT TTAAACCAAT AGGCCGAAAT
    CGCAATTATA AACAAATTA GTCGAGTAAA AAATTGGTTA TCCGGCTTTA

701  CGGCAAAATC CCTATATAAT CAAAGAATA GACCGAGATA GGGTTGAGTG
    GCCGTTTATG GGAATATTA GTTTCCTTAT CTGGCTCTAT CCCAACTCAC

751  TTGTTCCAGT TTGGAACAAG AGTCCACTAT TAAAGAACGT GGACTCCAAC
    AACAAAGTCA AACCTTGTC TCAGGTGATA ATTTCTTGCA CTGAGGTTG

801  GTCAAAGGCG GAAAACCGT CTATCAGGGC GATGGCCCAC TACGAGAAC
    CAGTTTCCCG CTTTTTGCA GATAGTCCCG CTACCGGGTG ATGCTCTTGG

851  ATCACCCCTAA TCAAGTTTTT TGGGGTCGAG GTGCCGTAAA GACTAAATC
    TAGTGGGATT AGTCAAAAA ACCCCAGCTC CACGGCATTT CGTGATTTAG

          BanII
          ~~~~~

901  GGAACCCCTAA AGGAGCCCC CGATTTAGAG CTTGACGGGG AAAGCCGGCG
    CCTGGGGATT TCCCTCGGGG GCTAAATCTC GAACTGCCCC TTTCGGCCCG

951  AACGTGGCGA GAAAGGAAGG GAAGAAAGCG AAAGGAGCGG GCGTAGGGC
    TTGCACCGCT CTTTCCTTCC CTTCCTTCGC TTTCCTCGCC CGCGATCCCC

```

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

```

1001 GCTGGCAAGT GTAGCGGTCA CGCTGCGCGT AACCAACACA CCCGCCGCGC
      CGACCGTTCA CATGCCAGT GCGACGCGCA TTGGTGGTGT GGGCGGCGCG

      NheI
      ~~~~~
1051 TTAATGCGCC GCTACAGGGC GCGTGCTAGC CATGTGAGCA AAAGGCCAGC
      AATTACGGG CGATGTCCCG CGCACGATCG GTACACTCGT TTTCCGGTCCG

1101 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG
      TTTTCCGGTC CTTGGCATT TTTCCGGCGCA ACGACCGCAA AAAGGTATCC

1151 CTCCGCCCCC CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG
      GAGCGGGGG GACTGCTCGT AGTGTTTSTA GCTGCGAGTT CAGTCTCCAC

1201 GCGAAACCCG ACAGGACTAT AAAGATACCA GCGGTTTCCC CCTGGAAGCT
      CGCTTTGGGC TGTCTTGATA TTTCTATGGT CCGCAAAGG GGACCTTCGA

      BssSI
      ~~~~~
1251 CCCTCGTGCG CTCTCCTGTT CCGACCCCTGC CGCTTACCGG ATACCTGTCC
      GCGAGCACGC GAGAGGACAA GGCTGGGACG CGGAATGGCC TATGGACAGG

1301 GCCTTTCTCC CTTGCGGAAG CGTGCGCGCTT TCTCATAGCT CACGCTGTAG
      CGGAAAGAGG GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC

```

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

|      |             |             |             |            |             |
|------|-------------|-------------|-------------|------------|-------------|
| 1351 | GTATCTAGT   | TCGGTGTAGG  | TCGTTGCGCTC | CAAGCTGGGC | TGTGTGCACG  |
|      | CATAGAGTCA  | AGCCACATCC  | AGCAAGCGAG  | GTTCGACCCG | ACACACGTGC  |
| 1401 | AACCCCCCGT  | TCAGCCCCGAC | CGCTGCCGCTT | TATCCGGTAA | CTATCGTCTT  |
|      | TTGGGGGGCA  | AGTCGGGCTG  | GCGACGCCGA  | ATAGGCCATT | GATAGCAGAA  |
| 1451 | GAGTCCAACC  | CGGTAAGACA  | CGACTTATCG  | CCACTGGCAG | CAGCCACTGG  |
|      | CTCAGGTTGG  | GCCATTCTGT  | GCTGAATAGC  | GGTGACCGTC | GTCGGTGACC  |
| 1501 | TAAACAGGATT | AGCAGAGCGA  | GGTATGTAGG  | CGGTGCTACA | GAGTTCTTGA  |
|      | ATTGTCCTAA  | TCGTCTCGCT  | CCATACATCC  | GCCACGATGT | CTCAAGAACT  |
| 1551 | AGTGGTGGCC  | TAACTACGGC  | TACACTAGAA  | GAACAGTATT | TGGTATCTGC  |
|      | TCACCACCGG  | ATTGATGCCG  | ATGTGATCTT  | CTTGTCATAA | ACCATAGACG  |
| 1601 | GCTCTGCTGT  | AGCCAGTTAC  | CTTCGGAAAA  | AGAGTTGGTA | GCTCTTGATC  |
|      | CGAGACGACA  | TCGGTCAATG  | GAAGCCTTTT  | TCTCAACCAT | CGAGAACTAG  |
| 1651 | CGGCAAAACA  | ACCACCGCTG  | GTAGCGGTGG  | TTTTTTTGTT | TGCAAGCAGC  |
|      | GCCGTTTGTT  | TGGTGCGGAC  | CATCGCCACC  | AAAAAAACAA | ACGTTCTGTCG |
| 1701 | AGATTACGCG  | CAGAAAAAAA  | GGATCTCAAG  | AAGATCCTTT | GATCTTTTCT  |
|      | TCTAATGCGC  | GTCTTTTTTT  | CCTAGAGTTC  | TTCTAGGAAA | CTAGAAAAAGA |

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

```

1751  ACGGGGTCTG  ACGCTCAGTG  GAACGAAAC  TCACGTTAAG  GGATTTTGGT
      TGCCCCAGAC  TGCAGATCAC  CTTGCTTTTG  AGTGCAATTC  CCTAAAACCA

      BglII
      ~~~~~
1801  CAGATCTAGC  ACCAGGCGTT  TAAGGCACC  AATAACTGCC  TTAATAAAAT
      GTCTAGATCG  TGGTCCGCAA  ATTCCCCTGG  TTATTGACGG  AATTTTTTTA

1851  TACGCCCCCG  CCTGCCACTC  ATCGCAGTAC  TGTGTGAATT  CATTAAGCAT
      ATGCGGGGCG  GGACGGTGAG  TAGCGTCATG  ACAACATTAA  GTAAATTCGTA

1901  TCTGCCCGACA  TGGAAAGCCAT  CACAAACGGC  ATGATGAACC  TGAATCGCCA
      AGACGGCTGT  ACCTTCGGTA  GTGTTTGCCG  TACTACTTGG  ACTTAGCCGT

1951  GCGGCATCAG  CACCTTGTCG  CCTTGCGTAT  AATATTTGCC  CATAGTGAAA
      CGCCGTAAGC  GTGGAACAGC  GGAACGCATA  TTATAAACGG  GTATCACTTT

2001  ACGGGGGCGA  AGAAGTTGTC  CATATTGGCT  ACGTTTAAAT  CAAAACTGGT
      TGCCCCCGCT  TCTTCAACAG  GTATAACCGA  TGCAAATTTA  GTTTTGACCA

2051  GAAACTCACC  CAGGGATTGG  CTGAGACGAA  AAACATATTC  TCAATAAAC
      CTTTGAGTGG  GTCCCTAACC  GACTCTGCTT  TTTGTATAAG  AGTTATTG

```

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Figure 35: functional map and sequence of modular vector pCAL4 (continued)

|      |                                                                                                                   |
|------|-------------------------------------------------------------------------------------------------------------------|
| 2101 | CTTTAGGAA ATAGGCCAGG TTTTCACCGT AACACGCCAC ATCTTGCGAA<br>GAAATCCCTT TATCCGGTCC AAAAGTGGCA TTGTGCGGTG TAGAACGCTT   |
| 2151 | TATATGTGTA GAAACTGCCG GAAATCGTCG TGGTATTCAC TCCAGAGCGA<br>ATATACACAT CTTTGACGGC CTTTAGCAGC ACCATAAGTG AGGTCCTCGCT |
| 2201 | TGAAAACGTT TCAGTTTGCT CATGGAAAC GGTGTAACAA GGTGAACAC<br>ACTTTTGCAA AGTCAAACGA GTACCTTTTG CCACATTGTT CCCACTTGTG    |
| 2251 | TATCCCATAT CACCAGCTCA CCGTCTTTCA TTGCCATACG GAACTCCGGG<br>ATAGGGTATA GTGGTCGAGT GGCAGAAAGT AACGGTATGC CTTGAGGCC   |
| 2301 | TGAGCATTCA TCAGGCGGGC AAGAATGTGA ATAAAGGCCG GATAAACTT<br>ACTCGTAAGT AGTCCGCCCG TTCTTACACT TATTTCCGGC CTATTTTGAA   |
| 2351 | GTGCTTATTT TTCTTTACGG TCTTTAAAAA GGCCGTAATA TCCAGCTGAA<br>CACGAATAAA AAGAAATGCC AGAAATTTT CCGCATATAT AGGTCGACTT   |
| 2401 | CGGCTCTGTT ATAGGTACAT TGAGCAACTG ACTGAAATGC CTCAAAATGT<br>GCCAGACCAA TATCCATGTA ACTCGTTGAC TGACTTTACG GAGTTTAC    |
| 2451 | TCTTTACGAT GCCATTGGGA TATATCAACG GTGGTATATC CAGTGATTTT<br>AGAAATGCTA CCGTAACCCCT ATATAGTTGC CACCATATAG GTCACATAAA |

Figure 35: functional map and sequence of modular vector pCAL4 (continued)

```

2501  TTTCTCCATT  TTAGCTTCCT  TAGCTCCTGA  AAATCTCGAT  AACTCAAAA
      AAAGAGGTAA  AATCGAAGGA  ATCGAGGACT  TTTAGAGCTA  TTGAGTTT
2551  ATACGCCCGG  TAGTGATCTT  ATTCATTAT  GGTGAAAGTT  GGAACCTCAC
      TATGCGGGCC  ATCACTAGAA  TAAAGTAATA  CCACTTTCAA  CCTGGAGTG

      AatII
      ~~~~~
2601  CCGACGTCTA  ATGTGAGTTA  GCTCACTCAT  TAGGCACCCC  AGGCTTTACA
      GGCTGCAGAT  TACACTCAAT  CGAGTGAGTA  ATCCGTGGGG  TCCGAAATGT
2651  CTTTATGCTT  CCGGCTCGTA  TGTGTGTGG  AATTGTGAGC  GGATAACAAT
      GAAATACGAA  GGCCGAGCAT  ACAACACACC  TTAACACTCG  CCTATTGTTA

      XbaI   SphI
      ~~~~~
2701  TTCACACAGG  AAACAGCTAT  GACCATGATT  ACGAATTCTT  AGAGCATGCG
      AAGTGTGTCC  TTTGTGCGTA  CTGGTACTAA  TGCTTAAAGA  TCTCGTACGC

      EcoRI

2751  GGGGG
      CCCCC

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors

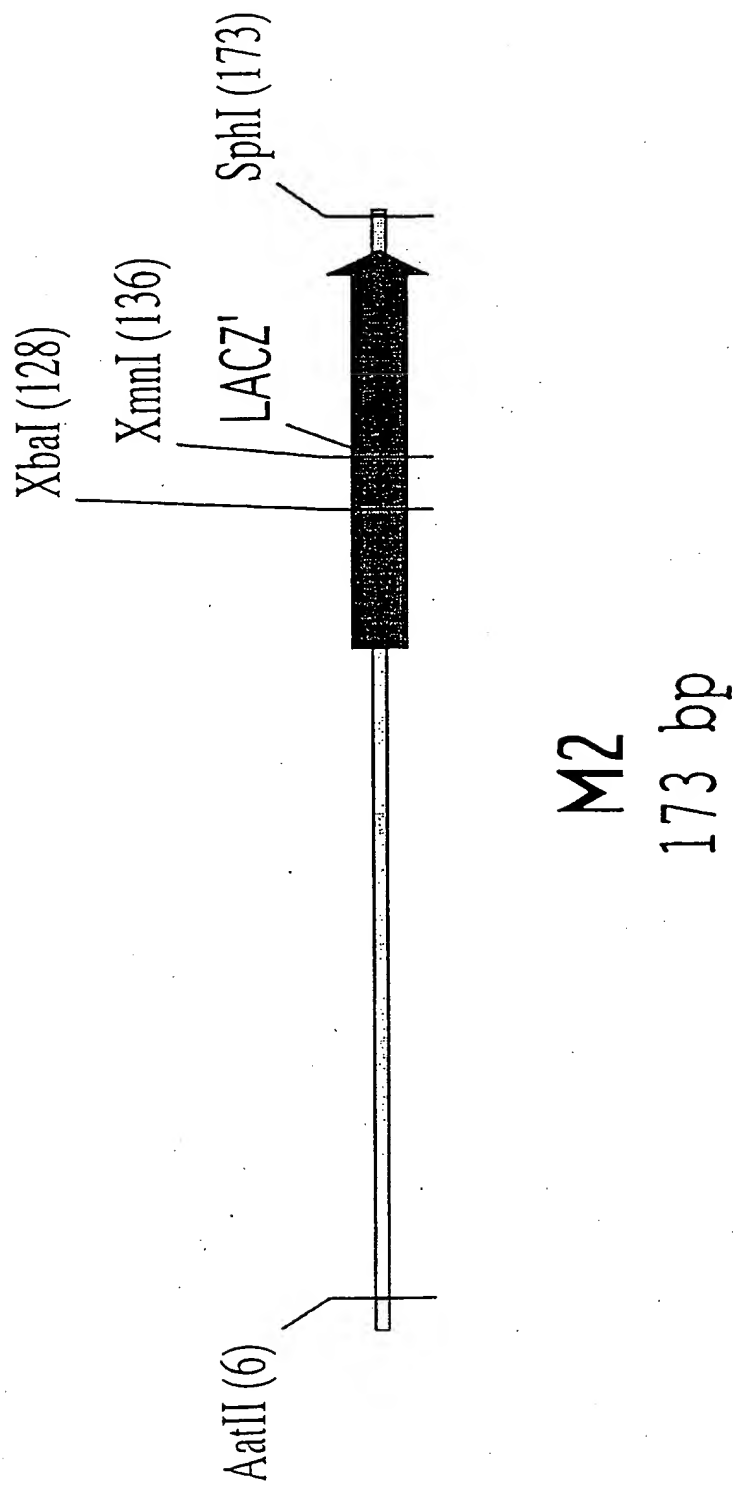


Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 2:

|     |             |            |            |            |            |
|-----|-------------|------------|------------|------------|------------|
|     | AatII       |            |            |            |            |
|     | ~~~~~       |            |            |            |            |
| 1   | GACGTCCTTAA | TGTGAGTTAG | CTCACTCATT | AGGCACCCCA | GGCTTTACAC |
|     | CTGCAGAAAT  | ACACTCAATC | GAGTGAGTAA | TCCGTGGGGT | CCGAAATGTG |
| 51  | TTTATGCTTC  | CGGCTCGTAT | GTTGTGTGGA | ATTGTGAGCG | GATAACAAAT |
|     | AAATACGAAG  | GCCGAGCATA | CAACACACCT | TAACACTCGC | CTATTGTTAA |
|     |             |            |            | XmnI       |            |
|     |             |            |            | ~~~~~      |            |
|     |             |            |            | XbaI       |            |
|     |             |            |            | ~~~~~      |            |
| 101 | TCACACAGGA  | AACAGCTATG | ACCATGTCTA | GAATAACTTC | GTATAATGTA |
|     | AGTGTGTCCT  | TTGTCGATAC | TGGTACAGAT | CTTATTGAAG | CATATTACAT |
|     |             |            |            | SphI       |            |
|     |             |            |            | ~~~~~      |            |
| 151 | CGCTATACGA  | AGTTATCGCA | TGC        |            |            |
|     | GGGATATGCT  | TCAATAGCGT | ACG        |            |            |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

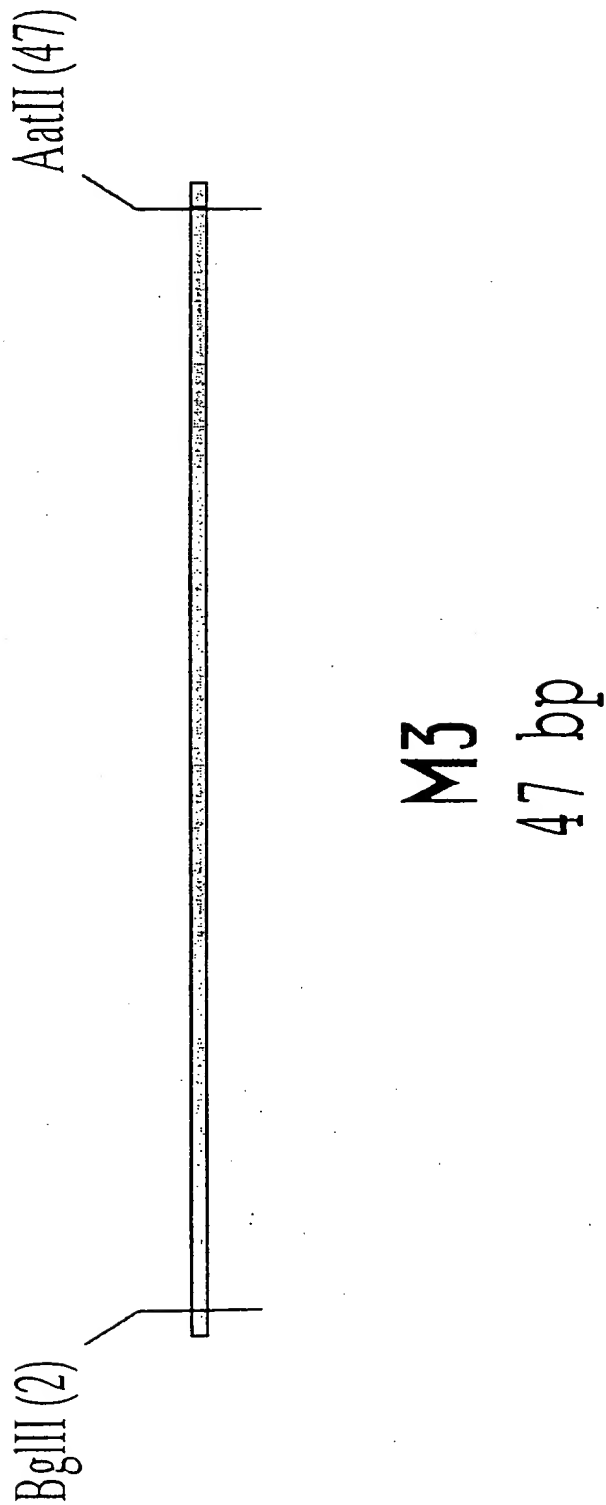


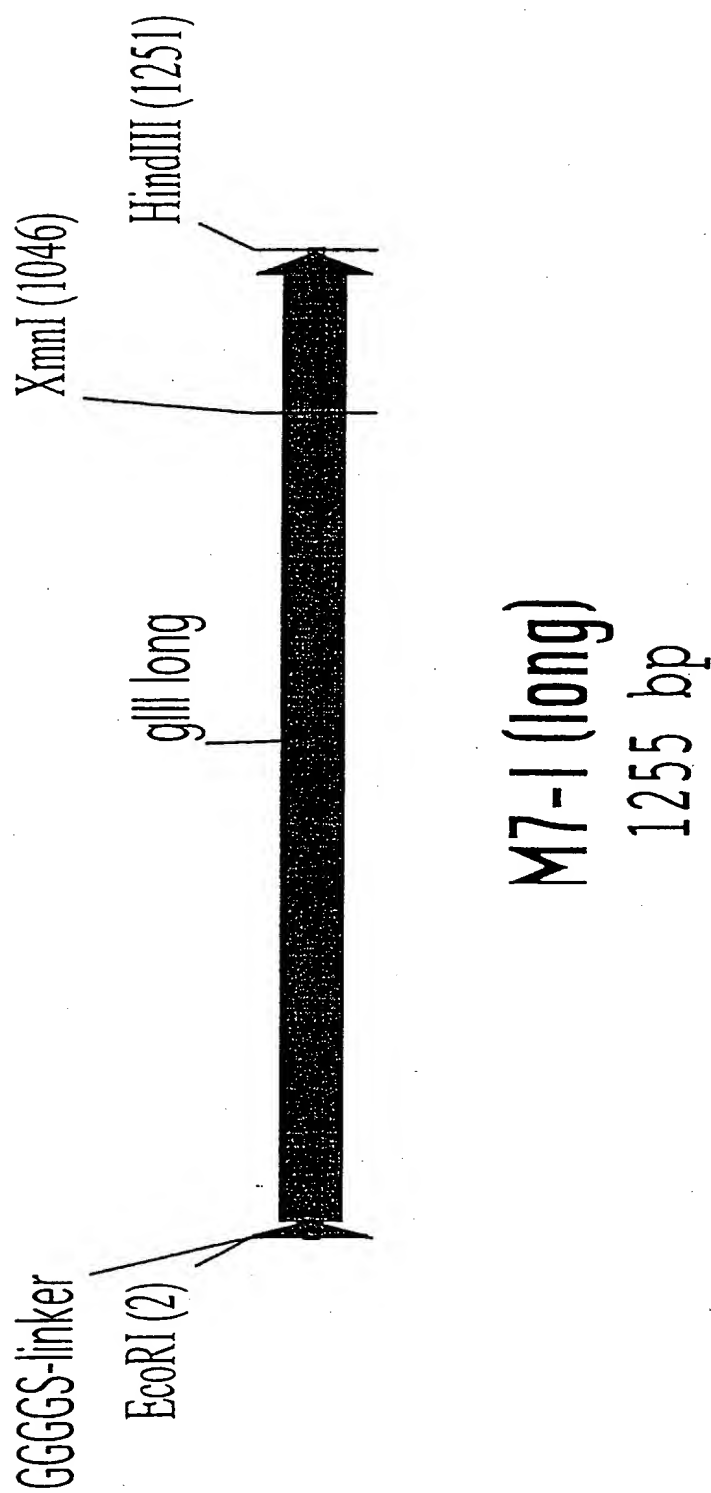
Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 3:

|   | BglII                                               | AatII |
|---|-----------------------------------------------------|-------|
|   | -----                                               | ----- |
| 1 | AGATCTCATA ACTTCGTATA ATGTATGCTA TACGAAGTTA TGACGTC |       |
|   | TCTAGAGTAT TGAAGCATAT TACATACGAT ATGCTTCAAT ACTGCAG |       |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

## M 7-I (long):

|     | EcORI                                                  |                                                         |
|-----|--------------------------------------------------------|---------------------------------------------------------|
|     | -----                                                  |                                                         |
| 1   | GAATTCGGTG GTGGTGGATC TGCGTGCGCT GAAACGGTTG AAAGTTGTTT | CTTAAGCCAC CACCACCTAG ACGCACGCGA CTTTGCCAAC TTTCACAAA   |
| 51  | AGCAAAATCC CATAAGAAA ATTCATTAC TAACGTCTGG AAAGACGACA   | TCGTTTTAGG GTATGTCTTT TAAGTAAATG ATTCGAGACC TTCTGCTGT   |
| 101 | AACTTTTAGA TCGTTACGCT AACTATGAGG GCTGTCTGTG GAATGCTACA | TTTGAAATCT AGCAATGCGA TTGATACTCC CGACAGACAC CTTACGATGT  |
| 151 | GGCGTTGTAG TTTGTACTGG TGACGAAACT CAGTGTTACG GTACATGGGT | CCGCAACATC AAACATGACC ACTGCTTTGA GTCACAAATGC CATGTACCCA |
| 201 | TCCTATTGGG CTTGCTATCC CTGAAAATGA GGGTGGTGGC TCTGAGGGTG | AGGATAACCC GAACGATAGG GACTTTTACT CCCACCAACCG AGACTCCCAC |
| 251 | GCGGTTCTGA GGGTGGCGGT TCTGAGGGTG GCGGTACTAA ACCTCCTGAG | CGCCAAGACT CCCACCGCCA AGACTCCCAC CGCCATGATT TGGAGGACTC  |
| 301 | TACGGTGATA CACCTATTCC GGGCTATACT TATATCAACC CTCTCGACGG | ATGCCACTAT GTGGATAAGG CCCGATATGA ATATAGTTGG GAGAGCTGCC  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```
351  CACTTATCCG CCTGGTACTG AGCAAAACCC CGCTAATCCT AATCCTTCTC
    GTGAATAGGC GGACCATGAC TCGTTTGGG GCGATTAGGA TTAGGAAGAG
401  TTGAGGAGTC TCAGCCCTCTT AATACTTTCA TGTTTCAGAA TAATAGGTTC
    AACTCCCTCAG AGTCGGAGAA TTATGAAAGT ACAAGTCTT ATTATCCAAG
451  CGAAATAGGC AGGGGGCATT AACTGTTTAT ACGGGCACTG TTAATCAAGG
    GCTTTATCCG TCCCCCGTAA TTGACAAATA TGCCCGTGAC AATGAGTTCC
501  CACTGACCCC GTTAAACTT ATTACCAGTA CACTCCTGTA TCATCAAAAG
    GTGACTGGGG CAATTTTGAA TAATGGTCAT GTGAGGACAT AGTAGTTTTC
551  CCATGTATGA CGCTTACTGG AACGGTAAAT TCAGAGACTG CGCTTTCCAT
    GTACATACT GCGAATGACC TTGCCATTTA AGTCTCTGAC GCGAAAGGTA
601  TCTGGCTTTA ATGAGGATTT ATTTGTTTGT GAAATATCAAG GCCAATCGTC
    AGACCGAAAT TACTCCCTAAA TAAACAAACA CTTATAGTTC CGGTTAGCAG
651  TGACCTGCCT CAACCTCCTG TCAATGCTGG CGGCGGCTCT GTGGTGGTT
    ACTGGACGGA GTTGGAGGAC AGTTACGACC GCCGCCGAGA CCACCACCAA
701  CTGGTGGCGG CTCTGAGGGT GGTGGCTCTG AGGGTGGCGG TTCTGAGGGT
    GACCACCGCC GAGACTCCCA CCACCGAGAC TCCCACCGCC AAGACTCCCA
```

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

751  GCGGGCTCTG AGGAGGCGG TTCCGGTGGT GGCTCTGGTT CCGGTGATTT CCGGTGATTT
    CCGCCGAGAC TCCCTCCGCC AAGGCCACCA CCGAGACCAA GGCCACTAAA GGCCACTAAA

801  TGATTATGAA AAGATGGCAA ACGCTAATAA GGGGGCTATG ACCGAAAATG ACCGAAAATG
    ACTAATACTT TTCTACCGTT TCGGATTATT CCCCCGATAC TGGCTTTTAC TGGCTTTTAC

851  CCGATGAAAA CGCGCTACAG TCTGACGCTA AAGGCAAACT TGATTCTGTC TGATTCTGTC
    GGCTACTTTT GCGCGATGTC AGACTGCCGAT TTCCGTTTGA ACTAAGACAG ACTAAGACAG

901  GCTACTGATT ACGGTGCTGC TATCGATGGT TTCATTGGTG ACGTTTCCGG ACGTTTCCGG
    CGATGACTAA TGCCACGACG ATAGCTACCA AAGTAACCAC TGCAAAGGCC TGCAAAGGCC

951  CCTTGCTAAT GGTAATGGTG CTA CTGGTGA TTTTGCTGGC TCTAATTCCC TCTAATTCCC
    GGAACGATTA CCATTACCAC GATGACCCACT AAAACGACCG AGATTAAGGG AGATTAAGGG

                                XmnI
                                ~~~~~

1001 AAATGGCTCA AGTCGGTGAA GGTGATAATT CACCTTTAAT GAATAATTTC GAATAATTTC
    TTTACCGAGT TCAGCCACTT CCACTATTAA GTGGAAATTA CTTATTAAAG CTTATTAAAG

1051 CGTCAATATT TACCTTCCAT CCCTCAATCG GTTGAATGTC GCCCTTTTGT GCCCTTTTGT
    GCAGTTATAA ATGGAAGGTA GGGAGTTAGC CAACTTACAG CCGGAAAACA CCGGAAAACA

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |            |            |            |            |            |
|------|------------|------------|------------|------------|------------|
| 1101 | CTTTGGCGCT | GGTAAACCCT | ATGAATTTTC | TATTGATTGT | GACAAAATAA |
|      | GAAACCGCGA | CCATTGGGA  | TACTTAAAG  | ATAACTAACA | CTGTTTATT  |
| 1151 | ACTTATTCCG | TGGTGTCTTT | GCGTTCTTT  | TATATGTTGC | CACCTTTATG |
|      | TGAATAAGGC | ACCACAGAAA | CGCAAAGAAA | ATATACAACG | GTGGAATAC  |
|      |            |            |            |            | HindIII    |
| 1201 | TATGTATTTT | CTACGTTTGC | TAACATACTG | CGTAATAAGG | AGTCTTGATA |
|      | ATACATAAAA | GATGCAAACG | ATTGTATGAC | GCATTATTCC | TCAGAACTAT |
|      |            |            |            |            | HindI      |
|      |            |            |            |            | ~~~        |
| 1251 |            |            |            |            | AGCTT      |
|      |            |            |            |            | TCGAA      |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

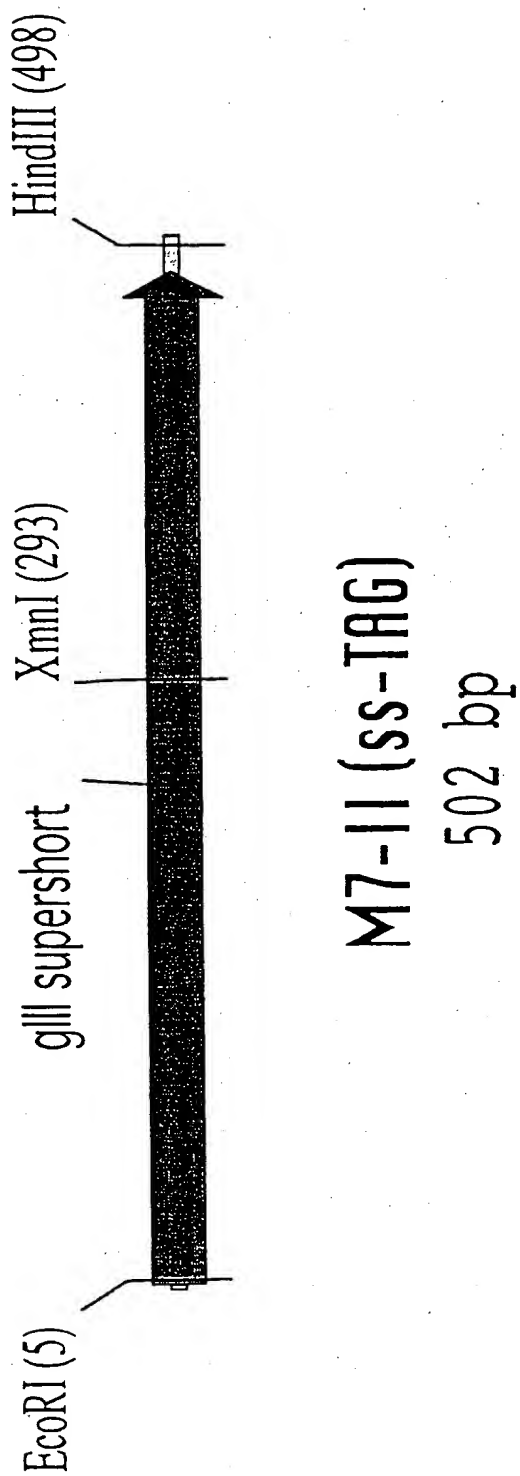


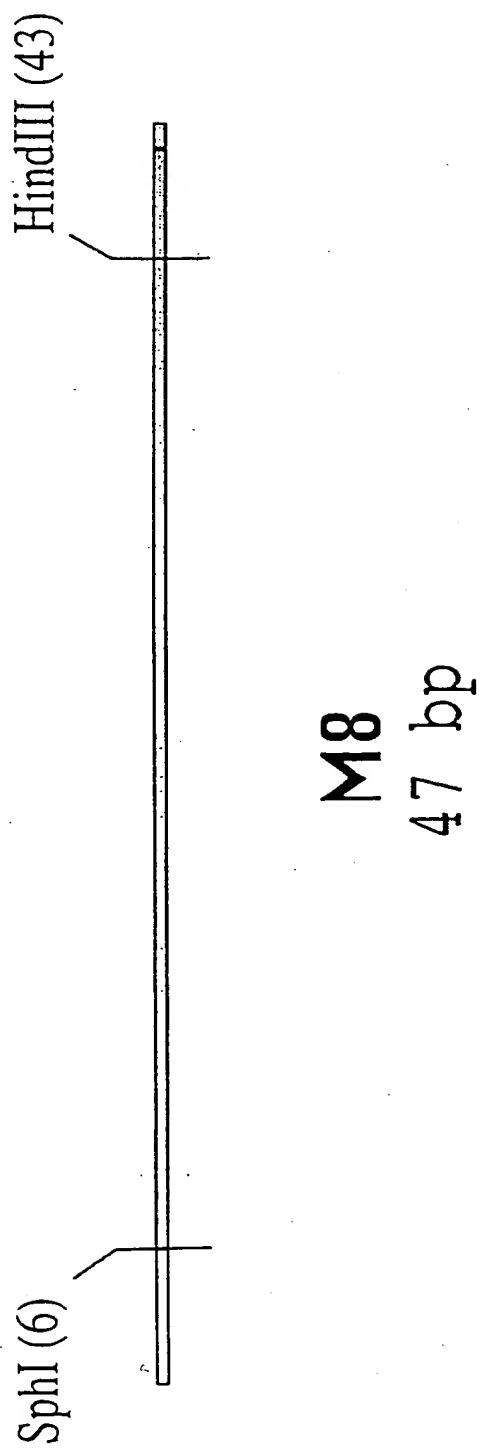
Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

| M 7-II (ss-TAG) : |                                                                                                                    |
|-------------------|--------------------------------------------------------------------------------------------------------------------|
| ECORI             |                                                                                                                    |
| ~~~~~             |                                                                                                                    |
| 1                 | CGGGAATTCTG GAGGCGGTTC CCGTGGTGGC TCTGGTTCCG GTGATTTTGA<br>GCCCTTAAGC CTCGCGCCAAG GCCACCACCG AGACCAAGGC CACTAAAACT |
| 51                | TTATGAAAAG ATGGCAAACG CTAATAAGGG GGCTATGACC GAAAATGCCG<br>AATACTTTC TACCGTTTGC GATTATTCCC CCGATACTGG CTTTACGGC     |
| 101               | ATGAAAACGC GCTACAGTCT GACGCTAAAG GCAAACCTTG TCTGTGCGCT<br>TACTTTTGCG CGATGTCAGA CTGCGATTTC CGTTTGAAC T AAGACAGCGA  |
| 151               | ACTGATTACG GTGCTGCTAT CGATGGTTTC ATTGGTGACG TTCCGGGCCT<br>TGACTAATGC CACGACGATA GCTACCAAAG TAACCACTGC AAAGGCCGGA   |
| 201               | TGCTAATGGT AATGGTGCTA CTGGTGATTT TGCTGGCTCT AATCCCCAA<br>ACGATTACCA TTACCACGAT GACCACCTAAA ACGACCGAGA TTAAGGGTTT   |
| XmnI              |                                                                                                                    |
| ~~~~~             |                                                                                                                    |
| 251               | TGGCTCAAGT CCGTGACGGT GATAATTCAC CTTTAATGAA TAATTTCCGT<br>ACCGAGTCA GCCACTGCCA CTATTAGTG GAAATTACTT ATTAAGGCA      |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|     |            |            |            |             |             |
|-----|------------|------------|------------|-------------|-------------|
| 301 | CAATATTAC  | CTCCCTCCC  | TCAATCGGT  | GAATGCGCC   | CTTTGTCTT   |
|     | GTTATAAATG | GAAGGAGGG  | AGTAGCCAA  | CTTACAGCGG  | GAAAACAGAA  |
| 351 | TGGCGCTGGT | AAACCATATG | AATTTCTAT  | TGATTGTGAC  | AAAATAAACT  |
|     | ACCGCGACCA | TTTGGTATAC | TTAAAGATA  | ACTAACACTG  | TTTTATTGA   |
| 401 | TATCCGTGG  | TGCTTTGCG  | TTTCTTTTAT | ATGTTGCCAC  | CTTTATGTAT  |
|     | ATAAGGCACC | ACAGAAACGC | AAAGAAAATA | TACAACGGTG  | GAAATACATA  |
|     |            |            |            | HindIII     | ----        |
| 451 | GTATTTTCTA | CGTTTGCTAA | CATACTGCGT | AATAAGGAGT  | CTTGATAAGC  |
|     | CATAAAAGAT | GCAACGATT  | GTATGACGCA | TTATTCCCTCA | GAACTATTCCG |
| 501 | Hi         | ~          | TT         | AA          |             |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 8:

|   | SphI                                                | HindIII |
|---|-----------------------------------------------------|---------|
|   | ~~~~~                                               | ~~~~~   |
| 1 | GCATGCCATA ACTTCGTATA ATGTACGCTA TACGAAGTTA TAAGCTT |         |
|   | CGTACGGTAT TGAAGCATAT TACATGCGAT ATGCTTCAAT ATTCGAA |         |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

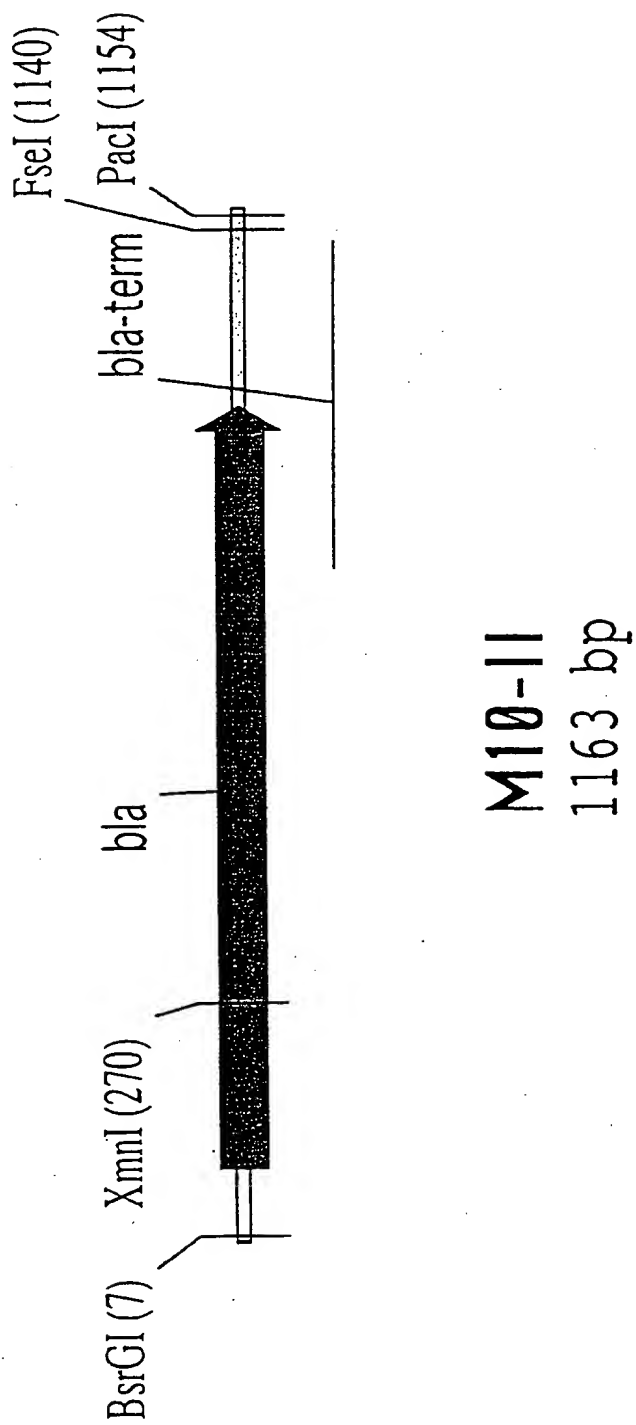


Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

## M 10-II:

BsrGI

~~~~~

```

1  GGGGTGTAC ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCTGATA
   CCCCACATG TAAGTTTATA CATAGCGGAG TACTCTGTTA TTGGGACTAT

51 AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTC
   TTACGAAGTT ATTATAACTT TTTCCCTTCTC ATACTCATAA GTTGTAAGG

101 GTGTCGCCCT TATTCCTTT TTTGCGGCAT TTTGCCCTTC TGTTTTGTCT
   CACAGCGGGA ATAAGGGAAA AAACGCCGTA AAACGGAAGG ACAAAAACGA

151 CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAGGATC AGTTGGGTGC
   GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTCCTAG TCAACCCACG

201 GCGAGTGGGT TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA
   CGCTCACCCA ATGTAGCTTG ACCTAGAGTT GTCGCCATTC TAGGAACTCT

```

XmnI

-----

```

251 GTTTCGCCC CGAAGAACGT TTCCAATGA TGAGCACTTT TAAAGTTCTG
   CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTCAAGAC

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

301  CTATGTGGCG  CCGTATTATC  CCGTATTGAC  GCCGGGCAAG  AGCAACTCGG
      GATACACCGC  GCCATAATAG  GGCATAACTG  CGGCCCGTTC  TCGTTGAGCC

351  TCGCCGCATA  CACTATTCTC  AGAATGACTT  GGTGAGTAC  TCACCAGTCA
      AGCGGCGTAT  GTGATAAGAG  TCTTACTGAA  CCAACTCATG  AGTGGTCAGT

401  CAGAAAAGCA  TCTTACGGAT  GGCATGACAG  TAAGAGAATT  ATGCAGTGCT
      GTCCTTTTCGT  AGAATGCCCTA  CCGTACTGTC  ATTCTCTTAA  TACGTCACGA

451  GCCATAACCA  TGAGTGATAA  CACTGCGGCC  AACTTACTTC  TGACAACGAT
      CCGTATTGGT  ACTCACTATT  GTGACGCCGG  TTGAATGAAG  ACTGTTGCTA

501  CGGAGGACCG  AAGGAGCTAA  CCGCTTTTTC  GCACAACATG  GGGATCATG
      GCCTCCTGGC  TTCCTCGATT  GCGGAAAAAA  CGTGTGTGAC  CCCTAGTAC

551  TAACTCGCCT  TGATCGTTGG  GAACCGGAGC  TGAATGAAGC  CATACCAAAC
      ATTGAGCGGA  ACTAGCAACC  CTTGGCCTCG  ACTTACTTCG  GTATGGTTTG

601  GACGAGCGTG  ACACCACGAT  GCCTGTAGCA  ATGGCAACAA  CGTTGCGCAA
      CTGCTCGCAC  TGTGGTGCTA  CGGACATCGT  TACCGTTGTT  GCAACGCGTT

651  ACTATTAACT  GGCGAACTAC  TTA CTCTAGC  TTCCCGGCAA  CAGTTAATAG
      TGATAATTGA  CCGCTTGATG  AATGAGATCG  AAGGGCCGTT  GTCAATTATC

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |   |             |
|------|---|-------------|
| 701  | ACTGGATGGA GCGGATATAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT  | CTCGGGCCCTT |
|      | TGACCTACCT CCGCCTATTT CAACGTCCTG GTGAAGACGC GAGCCGGGAA  |             |
| 751  | CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC  | AGCGTGGGTC  |
|      | GGCCGACCGA CCAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG   | TCGCACCCAG  |
| 801  | TCGCGGTATC ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCCGTATCG | TCCCCGTATCG |
|      | AGCGCCATAG TAACGTCGTG ACCCCGGTCT ACCATTCTGGG AGGCGATAGC | AGGCGATAGC  |
| 851  | TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA  | ACGAAATAGA  |
|      | ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT  | TGCTTTATCT  |
| 901  | CAGATCGCTG AGATAGGTGC CTCACTGATT AAGCATTTGG TAACTGTCAG  | TAACTGTCAG  |
|      | GTCTAGCGAC TCTATCCACG GAGTGACTAA TTCGTAACCC ATTGACAGTC  | ATTGACAGTC  |
| 951  | ACCAAGTTTA CTCATATATA CTTAGATTG ATTTAAAACT TCATTTTAA    | TCATTTTAA   |
|      | TGGTTCAAAT GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT  | AGTAAAAATT  |
| 1001 | TTTAAAAGGA TCTAGGTGAA GATCCTTTT GATAATCTCA TGACCAAAT    | TGACCAAAT   |
|      | AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTAA  | ACTGGTTTAA  |
| 1051 | CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA  | GTAGAAAAGA  |
|      | GGGAATTGCA CTCAAAAGCA AGTGACTCG CAGTCTGGG CATCTTTTCT    | CATCTTTTCT  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

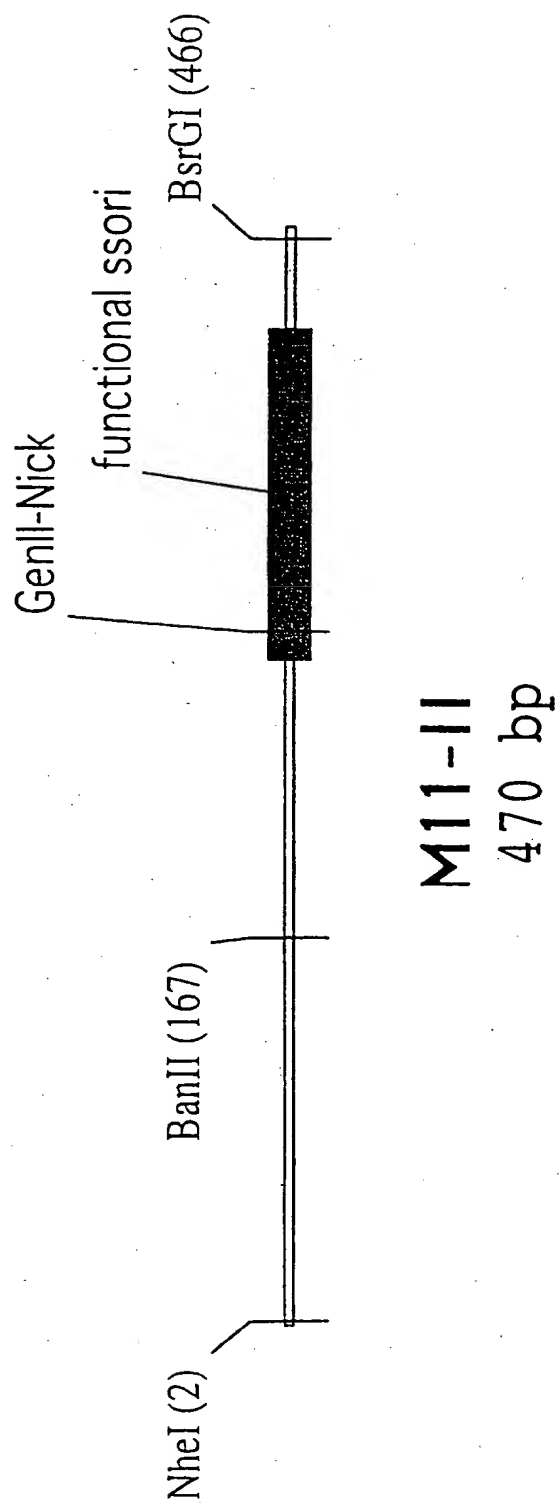
|      | FseI       |            | PacI      |            |
|------|------------|------------|-----------|------------|
|      | ~~~~~      |            | ~~~~~     |            |
| 1101 | TCAAAGGATC | TTCTTGAGAT | CCTTTTGAT | AATGGCCGGC |
|      | AGTTTCCTAG | AAGAACTCTA | GGAAAACTA | TTACCGGCCG |
|      |            |            |           | GGGGGGGAA  |

```

1151  PacI
      -----
      AATTAAGGG GGG
      TTAATTCCC CCC

```

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

## M11-II:

|     | NheI   |  |
|-----|--|--|
|     | ~~~~~  |  |
| 1   | GCTAGCACGC GCCCTGTAGC GCGGCATTAA GCGCGGCGGG TGTGGTGGTT | CGATCGTGCG CCGGACATCG CCGCGTAATT CCGCGCGCCC ACACCACCAA |
| 51  | ACGCGCAGCG TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT | TGCGCGTGCG ACTGGCGATG TGAACGTCG CCGGATCGCG GCGAGGAAA   |
| 101 | CGCTTTCCTC CCTTCCCTTC TCGCCACGTT CGCCGGCTTT CCCCCTCAAG | GCGAAAGAAG GGAAGGAAAG AGCGGTGCAA GCGGCCGAAA GGGCAGTTC  |
|     | BanII  |  |
|     | ~~~~~  |  |
| 151 | CTCTAAATCG GGGCTCCCT TTAGGGTTCC GATTAGTGC TTTACGGCAC   | GAGATTAGC CCCCAGGGA AATCCCAAGG CTAAATCACG AAATGCCGTG   |
| 201 | CTCGACCCCA AAAAATTGA TTAGGGTGAT GGTCTCGTA GTGGGCCATC   | GAGCTGGGT TTTTGAAC TATCCCACTA CCAAGAGCAT CACCCGGTAG    |
| 251 | GCCCTGATAG ACGGTTTTC GCCCTTTGAC GTTGAGTCC ACGTTCCTTA   | CGGGACTATC TGCCAAAAAG CCGGAAACTG CAACCTCAGG TGCAAGAAAT |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```
301  ATAGTGGACT CTTGTTCCAA ACTGGAACAA CACTCAACCC TATCTCGGTC
    TATCACCTGA GAACAAGGTT TGACCTTGTT GTGAGTTGGG ATAGAGCCAG

351  TATTCCTTTG ATTTATAAGG GATTTTGCCG ATTTCGGCCT ATTGGTTAAA
    ATAAGAAAAC TAAATATTCC CTAACAACGC TAAAGCCGGA TAACCAATT

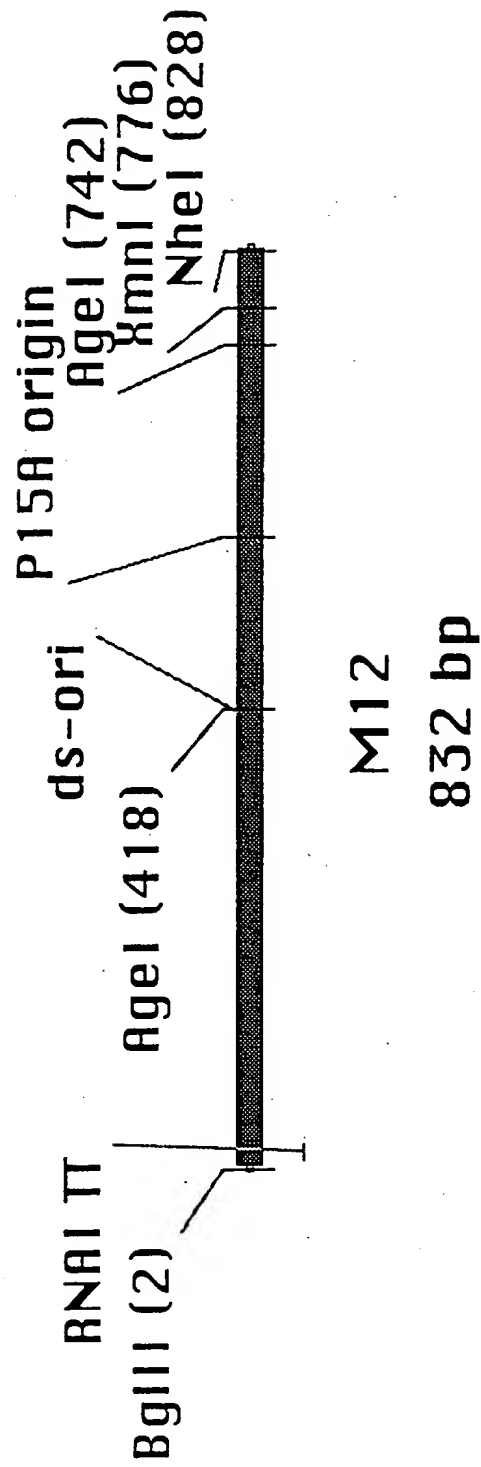
401  AAATGAGCTG ATTTAACAAA AATTTAACGC GAATTTTAAC AAAATATTAA
    TTTACTCGAC TAAATTGTTT TTAATTGCG CTTAAAAATTG TTTTATAATT

    BsrGI
    ~~~~~

451  CGTTACAAT TTCATGTACA
    GCAAAATGTA AAGTACATGT
```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|       |                          |                          |                          |                           |                          |
|-------|--------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| M 12: | BglII                    |                          |                          |                           |                          |
|       | ~~~~~                    |                          |                          |                           |                          |
| 1     | AGATCTAATA<br>TCTAGATTAT | AGATGATCTT<br>TCTACTAGAA | CTTGAGATCG<br>GAACTCTAGC | TTTTGGTCTG<br>AAAACCAGAC  | CGCGTAATCT<br>GCGCATTAGA |
| 51    | CTTGCTCTGA<br>GAACGAGACT | AAACGAAAAA<br>TTTGCTTTTT | ACCGCCTTGC<br>TGGCGGAACG | AGGGCGGTTT<br>TCCCGCCAAA  | TTCGTAGGTT<br>AAGCATCCAA |
| 101   | CTCTGAGCTA<br>GAGACTCGAT | CCAACCTCTT<br>GGTTGAGAAA | GAACCGAGGT<br>CTTGGCTCCA | AACTGGCTTG<br>TTGACCCGAAC | GAGGAGCGCA<br>CTCCTCGCGT |
| 151   | GTCACATAAA<br>CAGTGATTTT | CTTGTCCTTT<br>GAACAGGAAA | CAGTTTAGCC<br>GTCAAATCGG | TTAAACCGGCG<br>AATTGGCCGC | CATGACTTCA<br>GTACTGAAGT |
| 201   | AGACTAACTC<br>TCTGATTGAG | CTCTAAATCA<br>GAGATTTAGT | ATTACCAGTG<br>TAATGGTCAC | GCTGCTGCCA<br>CGACGACGGT  | GTGGTGCTTT<br>CACCACGAAA |
| 251   | TGCATGTCTT<br>ACGTACAGAA | TCCGGGTTGG<br>AGGCCCAACC | ACTCAAGACG<br>TGAGTTCCTG | ATAGTTACCG<br>TATCAATGGC  | GATAAGGCGC<br>CTATTCCGCG |
| 301   | AGCGGTCGGA<br>TCGCCAGCCT | CTGAACGGGG<br>GACTTGCCCC | GGTTCGTGCA<br>CCAAGCACGT | TACAGTCCAG<br>ATGTCAGGTC  | CTTGGAGCGA<br>GAACCTCGCT |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

351  ACTGCCCTACC CGGAACCTGAG TGTCAAGGCGT GGAATGAGAC AAACGCGGCGC
      TGACGGGATGG GCCTTGACTC ACAGTCCGCA CCTTACTCTG TTTGCGCGCCGG

      AgeI
      ~~~~~
401  ATAACAGCGG AATGACACCG GTAAACCGAA AGGCAGGAAC AGGAGAGCGC
      TATTGTGCGC TTA CTGTGGC CATTTGGCTT TCCGTCCTTG TCCTCTCGCG

451  AGGAGGGAGC CGCCAGGGGG AAACGCCCTGG TATCTTTATA GTCTGTCTCGG
      TCCTCCCTCG GCGGTCCCCC TTGCGGACC ATAGAAATAT CAGGACAGCC

501  GTTTCGCCAC CACTGATTG AGGTCAGAT TTCGTGATGC TTGTCAGGGG
      CAAAGCGGTG GTGACTAAAC TCGCAGTCTA AAGCACTACG AACAGTCCCC

551  GGCGGAGCCT ATGGAAAAC GGCTTTGCCG CGGCCCTCTC ACTTCCCCTGT
      CCGCCTCGGA TACCTTTTGG CCGAAACGGC GCCGGGAGAG TGAAGGAGACA

601  TAAGTATCTT CCTGGCATCT TCCAGGAAAT CTCCGCCCCCG TTCGTAAGCC
      ATTCATAGAA GGACCGTAGA AGGTCCCTTTA GAGCGGGGC AAGCATTCGG

651  ATTTCCGCTC GCCGCAGTCG AACGACCGAG CGTAGCGAGT CAGTGAGCGA
      TAAAGCGGAG CGGCGTCAGC TTGCTGGCTC GCATCGCTCA GTCACCTCGCT

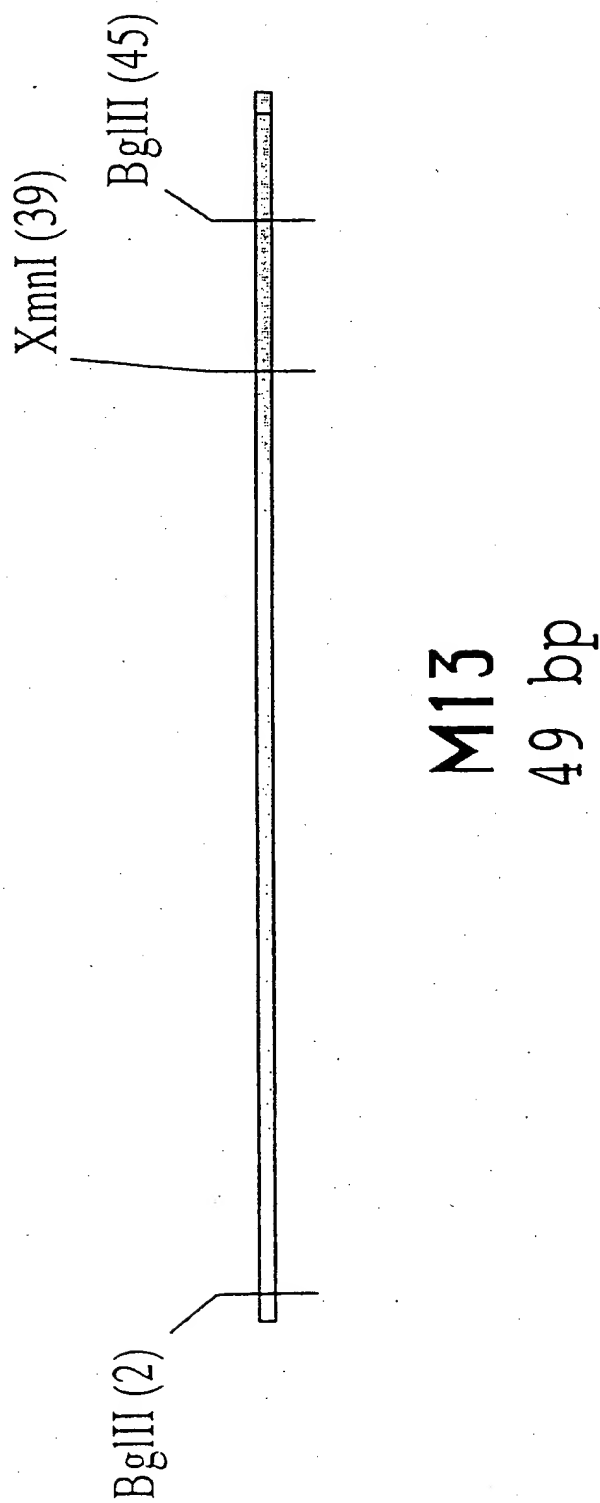
```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|     |                           |                           |                           |                          |                           |  |  |
|-----|---------------------------|---------------------------|---------------------------|--------------------------|---------------------------|--|--|
|     |                           | AgeI                      |                           |                          |                           |  |  |
|     |                           | ~~~~~                     |                           |                          |                           |  |  |
| 701 | GGAAGCGGAA<br>CCTTCGCCCTT | TATATCCCTGT<br>ATATAGGACA | ATCACATATT<br>TAGTGATAA   | CTGCTGACGC<br>GACGACTGGG | ACC GGTGCAG<br>TGGCCACGTC |  |  |
|     |                           |                           | XmnI                      |                          |                           |  |  |
|     |                           |                           | ~~~~~                     |                          |                           |  |  |
| 751 | CCTTTTTTTCT<br>GGAAAAAAGA | CCTGCCACAT<br>GGACGGTGTA  | GAAGCACTTC<br>CTTCGTGAAG  | ACTGACACCC<br>TGACTGTGGG | TCATCAGTGC<br>AGTAGTCACG  |  |  |
|     |                           |                           |                           |                          |                           |  |  |
|     |                           |                           | NheI                      |                          |                           |  |  |
|     |                           |                           | ~~~~~                     |                          |                           |  |  |
| 801 | CAACATAGTA<br>GTTGTATCAT  | AGCCAGTATA<br>TCGGTCATAT  | CACTCCGCTA<br>GTGAGGCCGAT | GC<br>CG                 |                           |  |  |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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**M 13:**

|   | BglII      | XmnI       | BglII     |
|---|------------|------------|-----------|
| 1 | AGATCTCATA | ATGTATGCTA | TTCAGATCT |
|   | TCTAGAGTAT | TACATACGAT | AAGTCTAGA |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

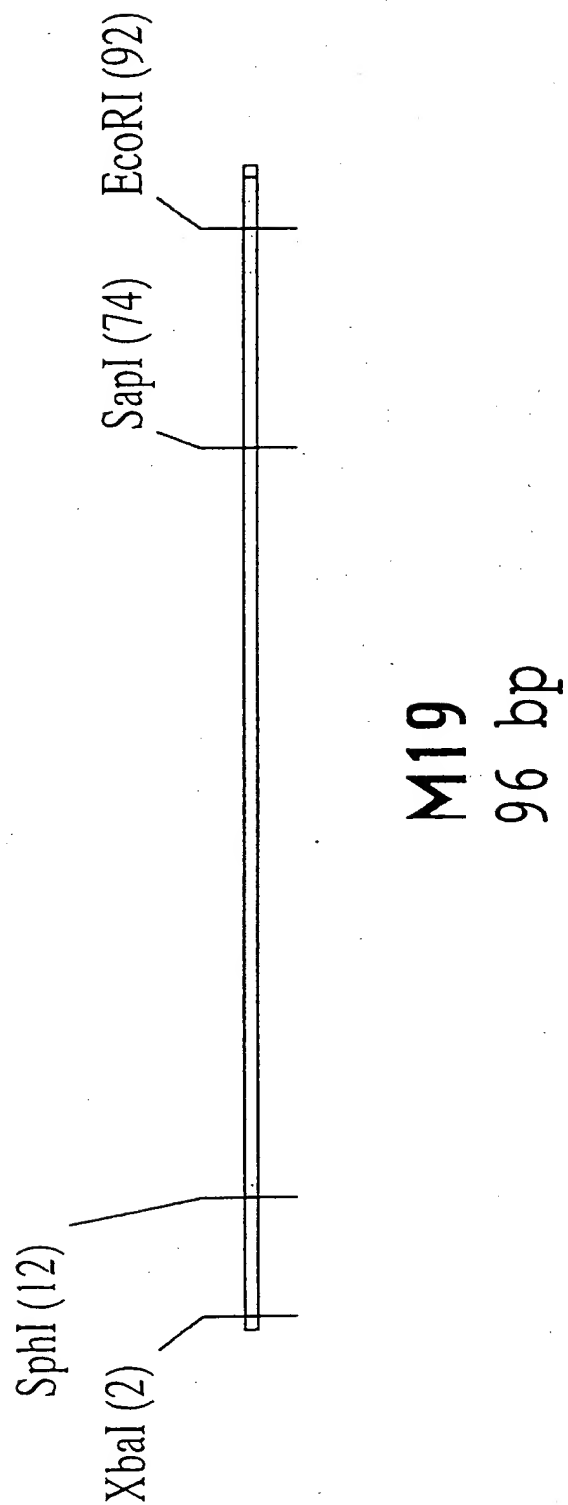


Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 19:

|    |            |             |                                  |
|----|------------|-------------|----------------------------------|
|    | XbaI       | SphI        |                                  |
|    | -----      |             |                                  |
| 1  | TCTAGAGCAT | GCGTAGGAGA  | AAATAAATG AAACAAAGCA CTATTGCACT  |
|    | AGATCTCGTA | CGCATCCCTCT | TTTATTTTAC TTTGTTTCGT GATAACGTGA |
|    |            | SapI        | ECORI                            |
|    |            | -----       | -----                            |
| 51 | GGCACTCTTA | CCGTTGCTCT  | TCACCCCTGT TACCAAAGCC GAATTC     |
|    | CCGTGAGAAT | GGCAACGAGA  | AGTGGGGACA ATGGTTTCGG CTTAAG     |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

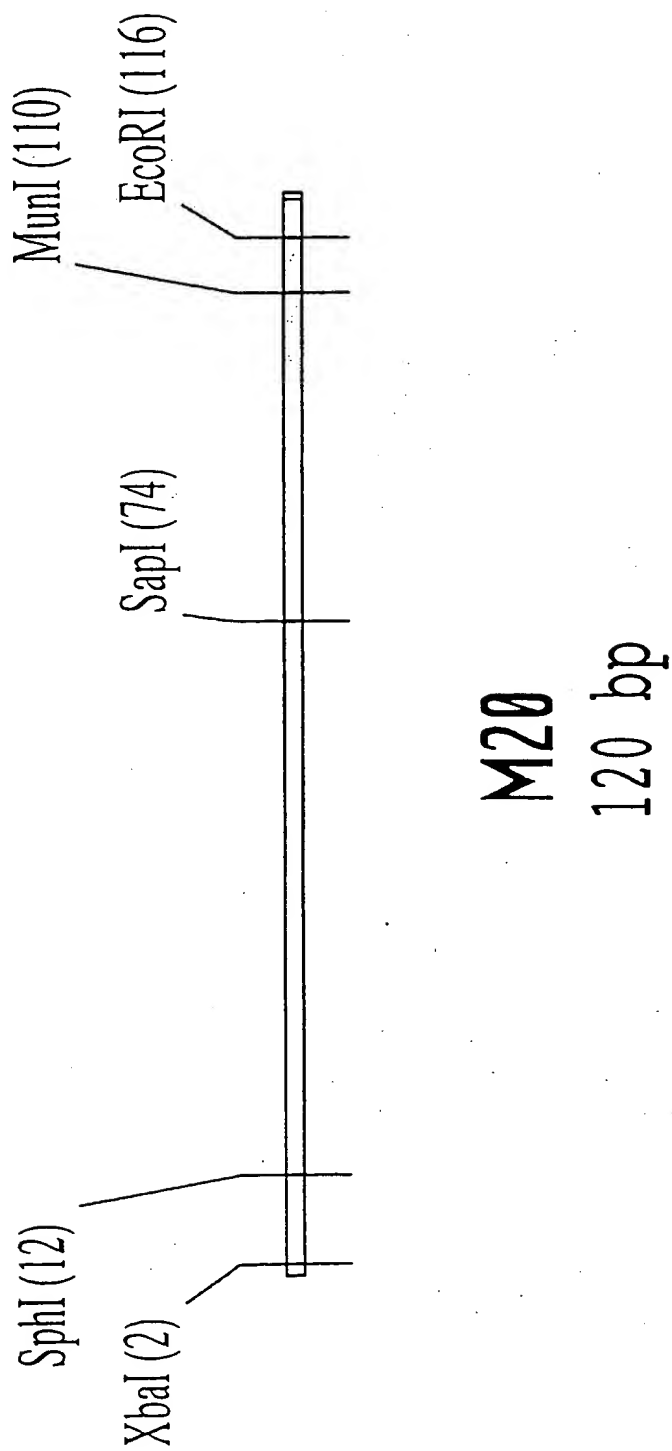


Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 20:

|     |             |            |                                  |
|-----|-------------|------------|----------------------------------|
|     | XbaI        | SphI       |                                  |
|     | ~~~~~       | ~~~~~      |                                  |
| 1   | TCTAGAGCAT  | GCGTAGGAGA | AAATAAAATG AACAAAGCA CTATTGCACT  |
|     | AGATCTCGTA  | CGCATCCTCT | TTTATTTTAC TTTGTTTCGT GATAACGTGA |
|     |             | SapI       |                                  |
|     |             | ~~~~~      |                                  |
| 51  | GGCACTCTTA  | CCGTTGCTCT | TCACCCCTGT TACCAAAGCC GACTACAAAG |
|     | CCGTGAGAAAT | GGCAACGAGA | AGTGGGGACA ATGGTTTCGG CTGATGTTTC |
|     |             | MunI       | EcoRI                            |
|     |             | ~~~~~      | ~~~~~                            |
| 101 | ATGAAGTGCA  | ATTGGAATC  |                                  |
|     | TACTTCACGT  | TAACCTTAAG |                                  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

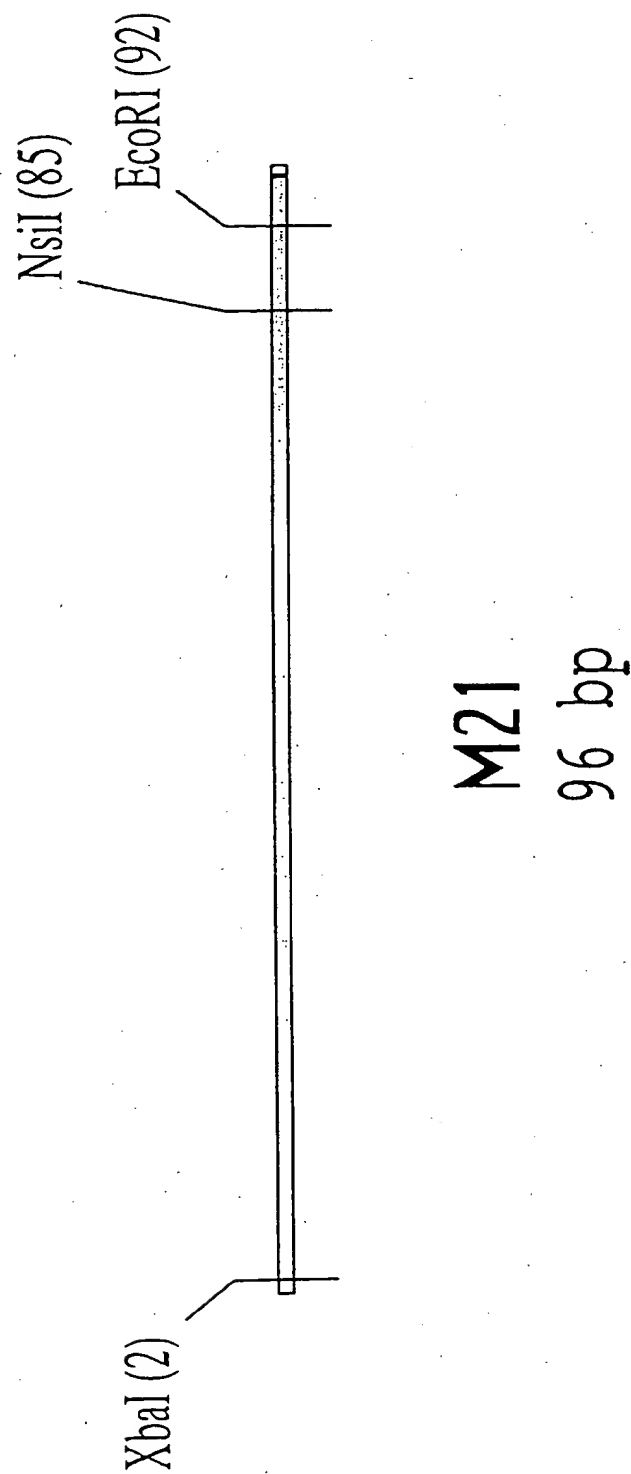


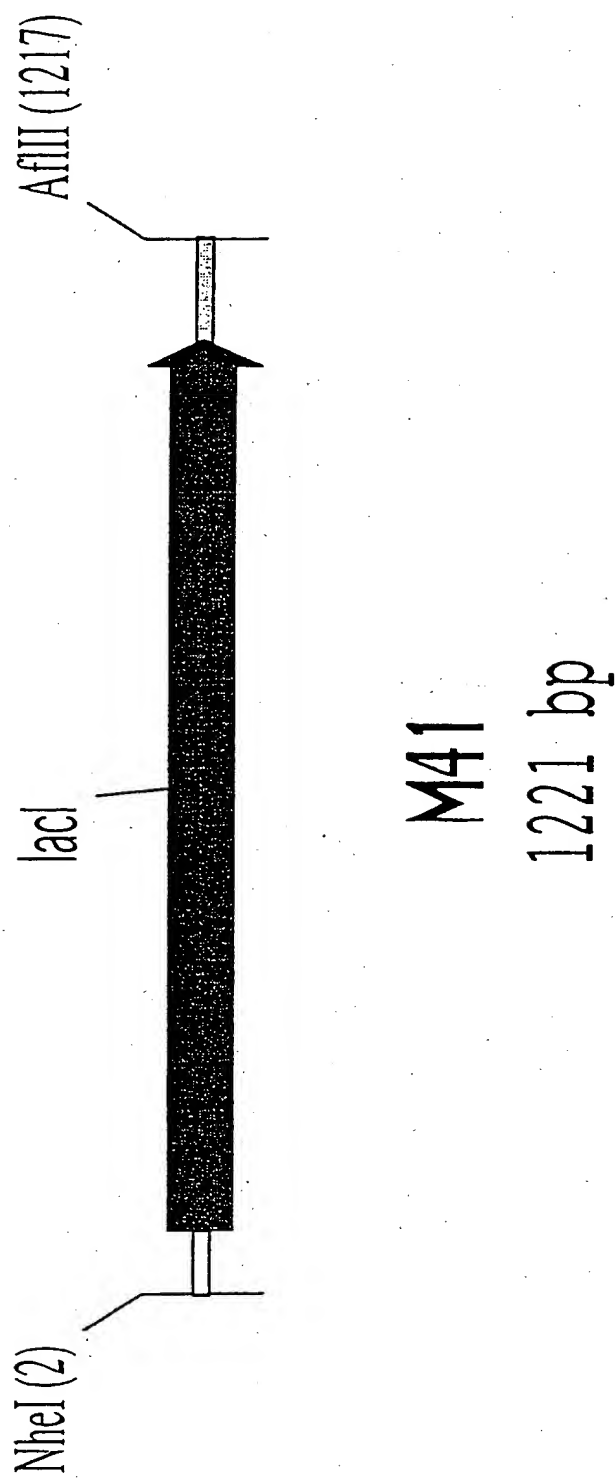
Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 21:

|    |  |       |       |
|----|--|-------|-------|
|    | XbaI   |       |       |
|    | -----  |       |       |
| 1  | TCTAGAGGTT GAGGTGATTT TATGAAAAAG AATATCGCAT TTCTTCTTGC |       |       |
|    | AGATCTCCAA CTCCACTAAA ATACTTTTTC TTATAGCCGT AAGAAGAACG |       |       |
|    |  | NsiI  | EcoRI |
|    |  | ----- | ----- |
| 51 | ATCTATGTTT GTTTTCTTA TTGCTACAAA TGCATACGCT GAATTC      |       |       |
|    | TAGATACAAG CAAAAAAGAT AACGATGTTT ACGTATGCCA CTTAAG     |       |       |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

M 41:

```

      NheI
      ~~~~
1  GCTAGCATCG AATGGCGCAA AACCTTTCGC GGTATGGCAT GATAGCGCCC
   CGATCGTAGC TTACCGCGTT TTGGAAGCGG CCATACCGTA CTATCGCGGG

51  GGAAGAGAGT CAATTCAGGG TGGTGAATGT GAAACCAGTA ACGTTATACG
   CCTTCTCTCA GTTAAGTCCC ACCACTTACA CTTTGGTCAT TGCAATATGC

101 ATGTCGCAGA GTATGCCGGT GTCTCTTATC AGACCGTTTC CCGCGTGGTG
   TACAGCGTCT CATACGGCCA CAGAGAATAG TCTGGCAAAG GCGCACCCAC

151 AACCAGGCCA GCCACGTTTC TCGGAAACG CGGGAAAAG TGGAAAGCGGC
   TTGGTCCGGT CCGTGCAAAG ACGCTTTTGC GCCCTTTTC ACCTTCGCCG

201 GATGGCGGAG CTGAATTACA TTCCTAACCG CGTGGCACAA CAACTGGCGG
   CTACCGCCTC GACTTAATGT AAGGATTGGC GCACCGTGT GTTGACCGCC

251 GCAAACAGTC GTTGCTGATT GGCGTTGCCA CCTCCAGTCT GGCCCTGCAC
   CGTTTGTGAG CAACGACTAA CCGCAACGGT GGAGGTCAGA CCGGGACGTG

301 GCGCCGTCGC AAATTGTCGC GCGGATTAAA TCTCGCGCCG ATCAACTGGG
   CGCGGCAGCG TTTAACAGCG CCGCTAATT AGAGCGCGGC TAGTTGACCC

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|     |            |            |             |            |             |
|-----|------------|------------|-------------|------------|-------------|
| 351 | TGCCAGCGTG | GTCGTGTCGA | TGGTAGAACG  | AAGCGGCGTC | GAAGCCTGTA  |
|     | ACGGTCGCAC | CAGCACAGCT | ACCATCTTGC  | TTCGCCGCAG | CTTCGGACAT  |
| 401 | AAGCGGCGGT | GCACAACTTT | CTCGCGCAAC  | GTGTCAGTGG | GCTGATTATT  |
|     | TTCGCCGCCA | CGTGTTAGAA | GAGCGCGTTG  | CACAGTCACC | CGACTAATAA  |
| 451 | AACTATCCGC | TGGATGACCA | GGATGCTATT  | GCTGTGGAAG | CTGCCCTGCAC |
|     | TTGATAGCG  | ACCTACTGGT | CCTACGATAA  | CGACACCTTC | GACGGACGTG  |
| 501 | TAATGTTCCG | GCGTTATTTC | TTGATGTCTC  | TGACCAGACA | CCCATCAACA  |
|     | ATTACAAGGC | CGCAATAAAG | AACTACAGAG  | ACTGGTCTGT | GGTAGTTGT   |
| 551 | GTATTATTTT | CTCCCATGAG | GACGGTACGC  | GACTGGGCGT | GGAGCATCTG  |
|     | CATAAATAAA | GAGGGTACTC | CTGCCATGCG  | CTGACCCGCA | CCTCGTAGAC  |
| 601 | GTCGCATTGG | GCCACCAGCA | AATCGCGCTG  | TTAGCTGGCC | CATTAAGTTC  |
|     | CAGCGTAACC | CGGTGGTCGT | TTAGCGCGAC  | AATCGACCCG | GTAATTCAAG  |
| 651 | TGCTCTCGCG | CGTCTGCGTC | TGGCTGGCTG  | GCATAAATAT | CTCACTCGCA  |
|     | ACAGAGCCGC | GCAGACGCAG | ACCGACCGAC  | CGTATTTATA | GAGTGAGCGT  |
| 701 | ATCAAATTCA | GCCGATAGCG | GAACGGGAAG  | GCGACTGGAG | TGCCATGTCC  |
|     | TAGTTTAAGT | CGGCTATCGC | CTTGCCCCTTC | CGCTGACCTC | ACGGTACAGG  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

751  GGTTTCAAC  AAACCATGCA  AATGCTGAAT  GAGGGCATCG  TTCCCACTGC
    CCAAAAGTTG  TTTGGTACGT  TTACGACTTA  CTCCTCGTAG  AAGGGTGACG

801  GATGCTGGTT  GCCAACGATC  AGATGGCGCT  GGGCGCAATG  CGTGCCATTA
    CTACGACCAA  CGGTTGCTAG  TCTACCGCGA  CCCGCCGTAC  GCACGGTAAT

851  CCGAGTCCGG  GCTGCGCGTT  GGTGCGGACA  TCTCGGTAGT  GGGATACGAC
    GGCTCAGGCC  CGACGCGCAA  CCACGCCCTGT  AGAGCCATCA  CCTATGCTG

901  GATACCGAGG  ACAGCTCATG  TTATATCCCG  CCGCTGACCA  CCATCAAACA
    CTATGGCTCC  TGTCGAGTAC  AATATAGGCG  GCGCACTGGT  GGTAGTTTGT

951  GGATTTTCGC  CTGCTGGGCG  AAACCAGCGT  GGACCGCTTG  CTGCAACTCT
    CCTAAAAGCG  GACGACCCCG  TTTGGTCGCA  CCTGGCGAAC  GACGTTGAGA

1001  CTCAGGGCCA  GCGGGTGAAG  GGCAATCAGC  TGTTGCCCGT  CTCACTGGTG
    GAGTCCCGGT  CCGCCACTTC  CCGTTAGTCG  ACAACGGGCA  GAGTGACCAC

1051  AAAAGAAAAA  CCACCCTGGC  TCCCAATACG  CAAACCGCCT  CTCCCCGCGC
    TTTTCTTTT  GGTGGGACCG  AGGTTATGCG  GTTTGGCGGA  GAGGGGCGCG

1101  GTTGGCCGAT  TCACTGATGC  AGCTGGCACG  ACAGGTTTCC  CGACTGGAAA
    CAACCGGCTA  AGTGACTACG  TCGACCGTGC  TGTCCAAAGG  GCTGACCTTT

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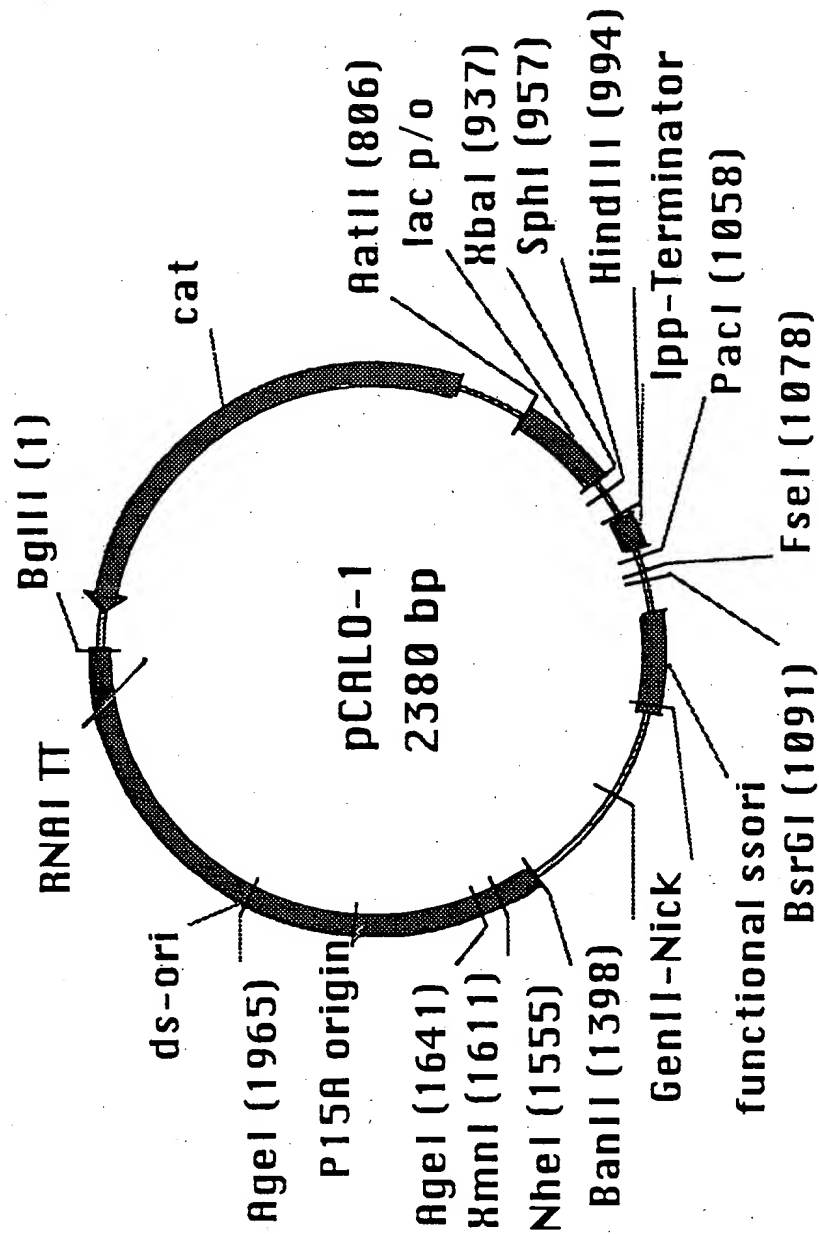
Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

1151 GCGGGCAGTG AGGCTACCCG ATAAAGCGG CTTCCTGACA GGAGGCCGTT  
CGCCCGTCAC TCCGATGGGC TATTTTCGCC GAAGGACTGT CCTCCGGCAA

AflII  
~~~~~

1201 TTGTTTGTGA GCCCACTTAA G  
AACAAACGT CGGTGAATT C

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)



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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

pCALO-1:  
 BglII  
 ~~~~~  
 1 GATCTAGCAC CAGGCGTTTA AGGCGACCAA TAACTGCCCTT AAAAAATTA  
 CTAGATCGTG GTCCGCAAT TCCCGTGGTT ATTGACGGAA TTTTTTTAAT  
 51 CGCCCCGCC TGCCACTCAT CGCAGTACTG TTGTAATTCA TTAAGCATTC  
 GCGGGCGGG ACGGTGAGTA GCGTCATGAC AACATTAAAGT AATTCCGTAAG  
 101 TGCCGACATG GAAGCCATCA CAAACGGCAT GATGAACCTG AATCGCCAGC  
 ACGGCTGTAC CTTCCGGTAGT GTTTGCCGTA CTACTTGGAC TTAGCGGTGG  
 151 GGATCAGCA CCTTGTCCG CCTTGTCCG TATTTGCCCA TAGTGAAAC  
 CCGTAGTCGT GGAACAGCGG AACGCATATT ATAAACGGGT ATCACTTTTG  
 201 GGGGGCGAAG AAGTTGTCCA TATTGGCTAC GTTTAAATCA AAAC TGGTGA  
 CCCCCGCTTC TTCAACAGGT ATAACCGATG CAAATTTAGT TTGACCACT  
 251 AACTCACCCA GGATTTGGCT GAGACGAAA ACATATTCTC AATAAACCCCT  
 TTGAGTGGGT CCTAACCGA CTCTGCTTTT TGTATAAGAG TTAATTTGGGA  
 301 TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CAGCCACAT CTGCGAATA  
 AATCCCTTTA TCCGGTCCAA AAGTGGCATT GTGCGGTGTA GAACGCTTAT

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```
351 TATGTGTAGA AACTGCCGGA AATCGTCGTG GTATTCACTC CAGAGCGATG
    ATACACATCT TTGACGGCCT TTAGCAGCAC CATAAGTGAG GTCTCGCTAC

401 AAAACGTTTC AGTTTGCTCA TGGAAAACGG TGTAACAAGG GTGAACACTA
    TTTTGCAAAG TCAAACGAGT ACCTTTTGCC ACATTGTTCC CACTTGTGAT

451 TCCCATATCA CCAGCTCACC GTCTTTCATT GCCATACGGA ACTCCGGGTG
    AGGTATAGT GGTCGAGTGG CAGAAAGTAA CGGTATGCCT TGAGGCCCAC

501 AGCATTCATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA TAAAACTTGT
    TCGTAAGTAG TCCGCCCGTT CTTACACTTA TTTCGGCCTT ATTTGAACA

551 GCTTATTTT CTTACGGTC TTTAAAAAGG CCGTAATATC CAGCTGAACG
    CGAATAAAAA GAAATGCCAG AAATTTTCC GCATTATAG GTCGACTTGC

601 GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT CAAAATGTTC
    CAGACCAATA TCCATGTAAC TCGTTGACTG ACTTTACGGA GTTTTACAAG

651 TTACGATGC CATTGGGATA TATCAACGGT GGTATATCCA GTGATTTT
    AAATGCTACG GTAACCCCTAT ATAGTTGCCA CCATATAGGT CACTAAAAAA

701 TCTCCATTT AGCTTCCTTA GCTCCTGAAA ATCTCGATAA CTCAAAAAAT
    AGAGGTAAAA TCGAAGGAAT CGAGGACTTT TAGAGCTATT GAGTTTTTA
```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

751  ACGCCCGGTA GTGATCTTAT TTCATTATGG TGAAGTTGG AACCTCACCC
    TGCGGGCCAT CACTAGAATA AAGTAATACC ACTTCAACC TTGGAGTGGG

    AatII
    ~~~~~
801  GACGCTAAT GTGAGTTAGC TCACTCATTA GGCACCCAG GCTTTACACT
    CTGCAGATTA CACTCAATCG AGTGAGTAAT CCGTGGGTC CGAAATGTGA

851  TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT
    AATACGAAGG CCGAGCATAC AACACACCTT AACACTCGCC TATTGTTAAA

    XbaI
    ~~~~~
901  CACACAGGAA ACAGCTATGA CCATGATTAC GAATTCTAG ACCCCCCCCC
    GTGTGTCCTT TGTCGATACT GGTAATAATG CTAAAGATC TGGGGGGGGG

    SphI
    ~~~~~
951  CGCATGCCAT AACTTCGTAT AATGTACGCT ATACGAAGTT ATAAGCTTGA
    GCGTACGGTA TTGAAGCATA TTACATGCCA TATGCTTCAA TATTCGAACT

1001 CCTGTGAAGT GAAAATGGC GCAGATTGTG CGACATTTT TTTGTCTGCC
    GGACACTTCA CTTTTTACCG CGTCTAACAC GCTGTAAAAA AACACAGCGG

    HindIII
    ~~~~~

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      | PacI   | FseI  | BsrGI |
|------|--|-------|-------|
|      | ~~~~~  | ~~~~~ | ~~~~~ |
| 1051 | GTTTAATTAA AGGGGGGGG GGGCCGGCCT GGGGGGGGT GTACATGAAA<br>CAAATTAAAT TCCCCCCCCC CCGGCCGGA CCCCCCCCCA CATGTACTTT    |       |       |
| 1101 | TTGTAAACGT TAATATTTG TTAAAAATTCG CGTTAAATTT TTGTTAAATC<br>AACATTTGCA ATTATAAAAC AATTTTAAGC GCAATTTAAA AACAAATTAG |       |       |
| 1151 | AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC CTTATAAATC<br>TCGAGTAAAA AATTGGTTAT CCGGCTTTAG CCGTTTTAGG GAATATTTAG |       |       |
| 1201 | AAAAGAATAG ACCGAGATAG GGTGAGTGT TGTTCAGTT TGGAAACAAGA<br>TTTTTCTTATC TGGCTCTATC CCAACTCACA ACAAGGTCAA ACCTTGTTCT |       |       |
| 1251 | GTCCACTATT AAAGAACGTG GACTCCAACG TCAAAGGGCG AAAAACCGTC<br>CAGGTGATAA TTTCTTGAC CTGAGGTTGC AGTTTCCCCG TTTTGTGGCAG |       |       |
| 1301 | TATCAGGCG ATGGCCCACT ACGAGAACCA TCACCCCTAAT CAAGTTTTT<br>ATAGTCCCCG TACCGGGTGA TGCTCTTGGT AGTGGGATTA GTTCAAAAAA  |       |       |
|      |  |       | BanII |
|      |  |       | ~~~~~ |
| 1351 | GGGTCGAGG TGCCGTAAAG CACTAAATCG GAACCCCTAAA GGGAGCCCCC<br>CCCCAGCTCC ACGGCATTTC GTGATTTAGC CTTGGGATTT CCCTCGGGGG |       |       |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |  |
|------|--|
| 1401 | GATTAGAGC TTGACGGGA AAGCCGGCGA ACGTGGCGAG AAAGGAAGGG<br>CTAAATCTCG AACTGCCCTT TTCGGCCGCT TGCACCGCTC TTTCCCTTCCC    |
| 1451 | AAGAAAGCGA AAGGAGCGG CGCTAGGCG CTGGCAAGTG TAGCGGTCAC<br>TTCTTTTCGCT TTCCTCGCC GCGATCCCGC GACCGTTCAC ATCGCCAGTG     |
| 1501 | GCTGCGGTA ACCACCACAC CCGCCGCGCT TAATGCCCGC CTACAGGGCG<br>CGACGCGCAT TGGTGGTGTG GCGGCGCGCA ATTACGCGGC GATGTCCCGC    |
| NheI |  |
| 1551 | CGTGCTAGCG GAGTGATAC TGGCTTACTA TGTGGCACT GATGAGGGTG<br>GCACGATCGC CTCACATATG ACCGAATGAT ACAACCGTGA CTACTCCCAC     |
| XmnI |  |
| 1601 | TCAGTGAAGT GCTTCATGTG GCAGGAGAAA AAAGGCTGCA CCGGTGCGTC<br>AGTCACTTCA CGAAGTACAC CGTCCCTCTTT TTTCCGACGT GGCCACGCAG  |
| 1651 | AGCAGAAATAT GTGATACAGG ATATATTCCG CTTCCCTCGCT CACTGACTCG<br>TCGTCTTATA CACTATGTCC TATATAAGGC GAAGGAGCGA GTGACTGAGC |
| 1701 | CTACGCTCGG TCGTTCGACT GCGGCGAGCG GAAATGGCTT ACGAACGGGG   |

AgeI

XmnI

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued).

|      |            |              |             |             |             |
|------|------------|--------------|-------------|-------------|-------------|
|      | GATGCGAGCC | AGCAAGCTGA   | CGCCGCTCGC  | CTTTACCGAA  | TGCTTGCCCC  |
| 1751 | CGGAGATTTC | CTGGAAGATG   | CCAGGAAGAT  | ACTTAACAGG  | GAAGTGAGAG  |
|      | GCCCTCTAAG | GACCTTCTAC   | GGTCCCTTCTA | TGAATTGTCC  | CTTCACTCTC  |
| 1801 | GGCCGCGGCA | AAGCCGTTTT   | TCCATAGGCT  | CCGCCCCCCCT | GACAAGCATC  |
|      | CCGGCGCCGT | TTCGGCAAAA   | AGGTATCCGA  | GGCGGGGGA   | CTGTTCTGTAG |
| 1851 | ACGAAATCTG | ACGCTCAAAT   | CAGTGGTGGC  | GAAACCCGAC  | AGGACTATAA  |
|      | TGCTTTAGAC | TGCGAGTTTA   | GTCACCAACCG | CTTTGGGCTG  | TCCTGATATT  |
| 1901 | AGATACCAGG | CGTTTCCCCC   | TGGCGGCTCC  | CTCCTGCGCT  | CTCCTGTCTC  |
|      | TCTATGGTCC | GCAAAGGGG    | ACCGCCGAGG  | GAGGACGCGA  | GAGGACAAAG  |
|      |            | AgeI         |             |             |             |
|      |            | ~~~~~        |             |             |             |
| 1951 | TGCCTTTCGG | TTTACC GG TG | TCATTCCGCT  | GTTATGGCCG  | CGTTTGTCTC  |
|      | ACGGAAAGCC | AAATGGCCAC   | AGTAAGGCGA  | CAATACCGGC  | GCAACACAGAG |
| 2001 | ATTCCACGCC | TGACACTCAG   | TTCCGGGGTAG | GCAGTTCGCT  | CCAAGCTGGA  |
|      | TAAGTGCGG  | ACTGTGAGTC   | AAGGCCCATC  | CGTCAAGCGA  | GGTTCGACCT  |
| 2051 | CTGTATGCAC | GAACCCCCCG   | TTCAGTCCGA  | CCGCTGCGCC  | TTATCCGGTA  |
|      | GACATACGTG | CTTGGGGGGC   | AAGTCAGGCT  | GGCGACGCGG  | AATAGGCCAT  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |            |            |            |            |            |       |
|------|------------|------------|------------|------------|------------|-------|
| 2101 | ACTATCGTCT | TGAGTCCAAC | CCGGAAGAC  | ATGCAAAAGC | ACCACTGGCA |       |
|      | TGATAGCAGA | ACTCAGGTG  | GGCCTTTCTG | TACGTTTTCG | TGGTGACCGT |       |
| 2151 | GCAGCCACTG | GTAATTGATT | TAGAGGAGTT | AGTCTTGAAG | TCATGCGCCG |       |
|      | CGTCGGTGAC | CATTAACTAA | ATCTCCTCAA | TCAGAACTTC | AGTACGCGGC |       |
| 2201 | GTTAAGGCTA | AACTGAAAGG | ACAAGTTTAA | GTGACTGCCG | TCCTCCAAGC |       |
|      | CAATTCCGAT | TTGACTTTCC | TGTTCAAAAT | CACTGACGCG | AGGAGGTTCC |       |
| 2251 | CAGTTACCTC | GGTTCAAAGA | GTTGGTAGCT | CAGAGAACCT | ACGAAAAACC |       |
|      | GTCAATGGAG | CCAAGTTTCT | CAACCATCGA | GTCTCTTGG  | TGCTTTTGG  |       |
| 2301 | GCCCTGCAAG | GCGGTTTTTT | CGTTTTCAGA | GCAAGAGATT | ACGCGCAGAC |       |
|      | CGGGACGTT  | CGCCAAAAAA | GCAAAAGTCT | CGTTCTCTAA | TGCGCGTCTG |       |
|      |            |            |            |            |            | BglII |
| 2351 | CAAAACGATC | TCAAGAAGAT | CATCTTATTA |            |            |       |
|      | GTTTTGCTAG | AGTTCTTCTA | GTAGAATAAT |            |            |       |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

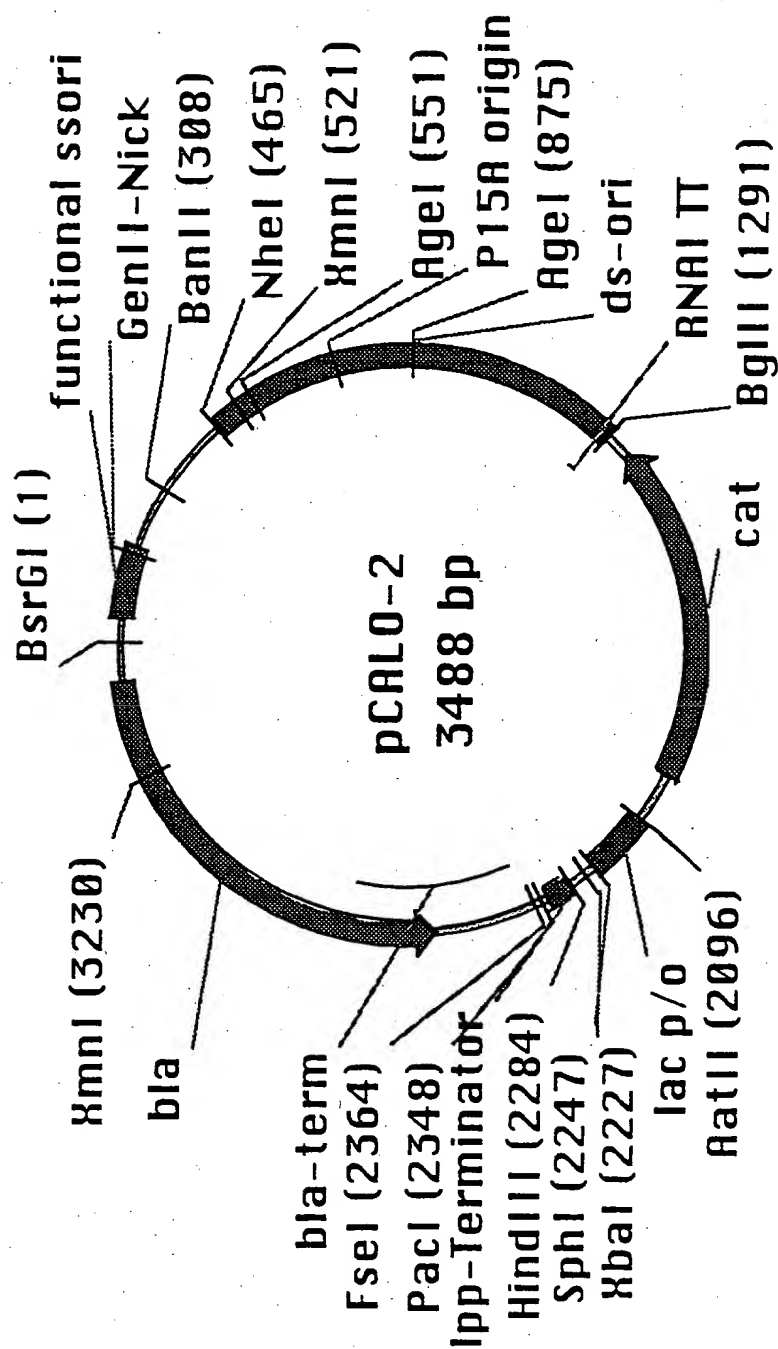




Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

pCALO-2:  
 BsrGI  
 ~~~~~

```

1  GTACATGAA  TTGTAAACGT  TAATATTTTG  TTAAAAATTCG  CGTTAAATTT
   CATGTA CTTT AACATTGCA  ATTATAAAAC  AATTTTAAGC  GCAATTTAAA

51  TTGTTAAATC  AGCTCATTTT  TTAACCAATA  GGCCGAAATC  GGCAAAATCC
   AACAAATTTAG  TCGAGTAAAA  AATTGGTTAT  CCGGCTTTAG  CCGTTTTAGG

101 CTTATAAATC  AAAAGAATAG  ACCGAGATAG  GGTGAGTGT  TGTTCAGTT
   GAATATTTAG  TTTTCTTATC  TGGCTCTATC  CCAACTCACA  ACAAGGTCAA

151 TGGAACAAGA  GTCCACTATT  AAAGAACGTG  GACTCCAACG  TCAAAGGGCG
   ACCTTGTTCT  CAGGTGATAA  TTTCTTGCAC  CTGAGGTTGC  AGTTTCCCGC

201 AAAAACCGTC  TATCAGGGCG  ATGGCCCACT  ACGAGAACCA  TCACCCTAAT
   TTTTGTGGCAG  ATAGTCCCGC  TACCGGGTGA  TGCTCTTGGT  AGTGGGATTA

251 CAAGTTTTTT  GGGGTCGAGG  TGCCGTAAAG  CACTAAATCG  GAACCCTAAA
   GTTCAAAAAA  CCCAGCTCC  ACGGCATTTC  GTGATTTAGC  CTTGGGATTT

                               BanII
                               ~~~~~

301 GGGAGCCCC  GATTAGAGC  TTACGGGGA  AAGCCGGCGA  ACGTGGCGAG
  
```

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

CCCTCGGGG CTAATCTCG AACTGCCCT TTCGGCGCT TGCACCGCTC
351 AAAGGAAGG AAGAAAGCGA AAGAGCGGG CGTAGGGCG CTGGCAAGTG
TTTCCCTTCCC TTCCTTCGCT TTCCTCGCC GCGATCCCGC GACCGTTCAC
401 TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG
ATCGCCAGTG CGACGCGCAT TGGTGGTGT GCGGCGCGA ATTACGCGGC
NheI
~~~~~
451 CTACAGGGCG CGTGCTAGCG GAGTGATAC TGGCTTACTA TGTGGCACT
GATGTCCCGC GCACGATCGC CTCACATATG ACCGAATGAT ACAACCGTGA
XmnI
~~~~~
501 GATGAGGGTG TCAGTGAAGT GCTTCATGTG GCAGGAGAAA AAAGGCTGCA
CTACTCCCGC AGTCACTTCA CGAAGTACAC CGTCCTCTTT TTCCGACGT
AgeI
~~~~~
551 CCGGTGCGTC AGCAGAATAT GTGATACAGG ATATATTCCG CTCCTCGCT
GGCCACGCAG TCGTCTTATA CACTATGTCC TATATAAGGC GAAGGAGCGA
601 CACTGACTCG CTACGCTCGG TCGTTCGACT GCGGCGAGCG GAAATGGCTT

```

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|     |             |            |            |             |             |
|-----|-------------|------------|------------|-------------|-------------|
|     | GTGACTGAGC  | GATGCGAGCC | AGCAAGCTGA | CGCCGCTCGC  | CTTTACCCGAA |
| 651 | ACGAACGGGG  | CGGAGATTTC | CTGGAAGATG | CCAGGAAGAT  | ACTTAACAGG  |
|     | TGCTTGCCCC  | GCCTCTAAAG | GACCTTCTAC | GGTCCCTTCTA | TGAATTGTCC  |
| 701 | GAAGTGAGAG  | GGCCGCGGCA | AAGCCGTTT  | TCCATAGGCT  | CCGCCCCCT   |
|     | CTTCACTCTC  | CCGGCGCCGT | TTCGGCAAAA | AGGTATCCGA  | GGCGGGGGA   |
| 751 | GACAAGCATC  | ACGAAATCTG | ACGCTCAAAT | CAGTGGTGGC  | GAAACCCGAC  |
|     | CTGTTTCGTAG | TGCTTTAGAC | TGCGAGTTTA | GTCACCAACCG | CTTTGGGCTG  |
| 801 | AGGACTATAA  | AGATACCAGG | CGTTTCCCCC | TGGCGGCTCC  | CTCCTGCCGT  |
|     | TCCTGATATT  | TCTATGGTCC | GCAAAGGGGG | ACCGCCGAGG  | GAGACGCCGA  |
|     |             |            | AgeI       |             |             |
|     |             |            | ~~~~~      |             |             |
| 851 | CTCCTGTTC   | TGCCTTTCGG | TTTACCGGTG | TCATTCCGCT  | GTTATGGCCG  |
|     | GAGACAAAGG  | ACGGAAAGCC | AAATGGCCAC | AGTAAGGCCA  | CAATACCCGC  |
| 901 | CGTTTGTC    | ATTCCACGCC | TGACACTCAG | TTCCGGGTAG  | GCAGTTCGCT  |
|     | GCAACACAGAG | TAAGGTGCGG | ACTGTGAGTC | AAGCCCATC   | CGTCAAGCGA  |
| 951 | CCAAGCTGGA  | CTGTATGCAC | GAACCCCCCG | TTCAGTCCGA  | CCGCTGCGCC  |
|     | GGTTCGACCT  | GACATACGTG | CTTGGGGGGC | AAGTCAGGCT  | GGCGACGCCG  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

```

1001 TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGAAAGAC ATGCAAAAGC
    AATAGGCCAT TGATAGCAGA ACTCAGGTTG GGCCTTTCTG TACGTTTTCG

1051 ACCACTGGCA GCAGCCACTG GTAATTGATT TAGAGGAGTT AGTCTTGAAG
    TGGTGACCGT CGTCGGTGAC CATTAACTAA ATCTCCTCAA TCAGAACTTC

1101 TCATGCGCCG GTTAAGGCTA AACTGAAAGG ACAAGTTTTA GTGACTGCCG
    AGTACGCGGC CAATTCCGAT TTGACTTTCC TGTCAAAAT CACTGACGCG

1151 TCCTCCAAGC CAGTTACCTC GGTCAAGA GTTGGTAGCT CAGAGAACCT
    AGGAGGTTTC GTCAATGGAG CCAAGTTTCT CAACCATCGA GTCTCTTGA

1201 ACGAAAACC GCCCTGCAAG GCGGTTTTTT CGTTTTCAGA GCAAGAGATT
    TGCTTTTTGG CGGGACGTTT CGCCAAAAA GCAAAGTCT CGTTCTCTAA

                                BglII
                                ~~~~~

1251 ACGCGCAGAC CAAAACGATC TCAAGAAGAT CATCTTATTA GATCTAGCAC
    TCGCGGTCCTG GTTTTGCTAG AGTTCTTCTA GTAGAAATAAT CTAGATCGTG

1301 CAGGCGTTTA AGGCAACCAA TAACTGCCTT AAAAAAATA CGCCCCGCC
    GTCCGCAAAT TCCCGTGTT ATTGACGGAA TTTTTTTAAAT GCGGGCGGG

```

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |             |             |            |             |             |
|------|-------------|-------------|------------|-------------|-------------|
| 1351 | TGCCACTCAT  | CGCAGTACTG  | TTGTAATTCA | TTAAGCATTC  | TGCCGACATG  |
|      | ACGGTGAGTA  | GCGTCATGAC  | AACATTAAGT | AATTCGTAAG  | ACGGCTGTAC  |
| 1401 | GAAGCCATCA  | CAAACGGCAT  | GATGAACCTG | AATCGCCAGC  | GGCATCAGCA  |
|      | CTTCGGTAGT  | GTTTGCCGTA  | CTACTTGGAC | TTAGCGGTCG  | CCGTAGTCGT  |
| 1451 | CCTTGTCGCC  | TTGCGTATAA  | TATTTGCCCA | TAGTAAAC    | GGGGCGAAG   |
|      | GGAACAGCGG  | AACGCATATT  | ATAAACGGGT | ATCACTTTTG  | CCCCCGCTTC  |
| 1501 | AAGTTGTCCA  | TATTGGCTAC  | GTTTAAATCA | AAACTGGTGA  | AACTCACCCA  |
|      | TTCAACAGGT  | ATAACCGATG  | CAAATTTAGT | TTTGACCACCT | TTGAGTGGGT  |
| 1551 | GGGATTGGCT  | GAGACGAAA   | ACATATTCTC | AATAAACCCCT | TTAGGGAAAT  |
|      | CCCTAACC GA | CTCTGCTTTT  | TGTATAAGAG | TTATTTGGGA  | AATCCCCTTTA |
| 1601 | AGGCCAGGTT  | TTACCCGTAA  | CACGCCACAT | CTTGCGAATA  | TATGTGTAGA  |
|      | TCCGGTCCAA  | AAGTGGCATT  | GTGCGGTGTA | GAACGCTTAT  | ATACACATCT  |
| 1651 | AACTGCCGGA  | AATCGTCGTG  | GTATTCACTC | CAGAGCGATG  | AAAACGTTTC  |
|      | TTGACGGCCT  | TTAGCAGCAC  | CATAAGTGAG | GTCTCGCTAC  | TTTTGCAAAG  |
| 1701 | AGTTTGCTCA  | TGGAACAACGG | TGTAACAAGG | GTGAACACTA  | TCCCATATCA  |
|      | TCAAACGAGT  | ACCTTTTGCC  | ACATTGTTCC | CACTTGTGAT  | AGGGTATAGT  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |             |            |            |            |            |
|------|-------------|------------|------------|------------|------------|
| 1751 | CCAGCTCACC  | GTCTTTCATT | GCCATACGGA | ACTCCGGGTG | AGCATTCATC |
|      | GGTCGAGTGG  | CAGAAAGTAA | CGGTATGCCT | TGAGGCCCAC | TCGTAAGTAG |
| 1801 | AGCGGGGCAA  | GAATGTGAAT | AAAGGCCGGA | TAAAACCTGT | GCTTATTTT  |
|      | TCCGCCCGTT  | CTTACACTTA | TTTCCGGCCT | ATTTGAACA  | CGAATAAAAA |
| 1851 | CTTTACGGTC  | TTTAAAAGG  | CCGTAATATC | CAGCTGAACG | GTCTGGTTAT |
|      | GAAATGCCAG  | AAATTTTCC  | GGCATATAG  | GTCGACTTGC | CAGACCAATA |
| 1901 | AGGTACATTG  | AGCAACTGAC | TGAAATGCCT | CAAAATGTTC | TTTACGATGC |
|      | TCCATGTAAC  | TCGTTGACTG | ACTTTACGGA | GTTTTACAAG | AAATGCTACG |
| 1951 | CATTGGGATA  | TATCAACGGT | GGTATATCCA | GTGATTTTTT | TCTCCATTTT |
|      | GTAACCCCTAT | ATAGTTGCCA | CCATATAGGT | CACTAAAAAA | AGAGGTAAAA |
| 2001 | AGCTTCCTTA  | GCTCCTGAAA | ATCTCGATAA | CTCAAAAAAT | ACGCCCCGTA |
|      | TCGAAGGAAT  | CGAGGACTTT | TAGAGCTATT | GAGTTTTTTA | TGCGGGCCAT |
|      |             |            |            | AatII      |            |
|      |             |            |            | ~~~~~      |            |
| 2051 | GTGATCTTAT  | TTCATATATG | TGAAAGTTGG | AACCTCACCC | GACGTCTAAT |
|      | CACTAGAATA  | AAGTAATACC | ACTTTC AAC | TTGGAGTGGG | CTGCAGATTA |
| 2101 | GTGAGTTAGC  | TCACTCATTA | GGCACCCAG  | GCTTTACACT | TTATGCTTCC |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |             |            |            |            |            |
|------|-------------|------------|------------|------------|------------|
|      | CACTCAATCG  | AGTGAGTAAT | CCGTGGGGTC | CGAAATGTGA | AATACGAAGG |
| 2151 | GGCTCGTATG  | TTGTGTGGAA | TTGTGAGCGG | ATAACAATTT | CACACAGGAA |
|      | CCGAGCATAC  | AACACACCTT | AACACTCGCC | TATTGTTAAA | GTGTGTCCTT |
|      |             | XbaI       |            | SphI       |            |
|      |             | ~~~~~      |            | ~~~~~      |            |
| 2201 | ACAGCTATGA  | CCATGATTAC | GAATTTCTAG | ACCCCCCCCC | CGCATGCCAT |
|      | TGTCGATACT  | GGTACTAATG | CTTAAAGATC | TGGGGGGGGG | GCGTACGGTA |
|      |             |            | HindIII    |            |            |
|      |             |            | ~~~~~      |            |            |
| 2251 | AAC TTCGTAT | AATGACGCT  | ATACGAAGTT | ATAAGCTTGA | CCTGTGAAGT |
|      | TTGAAGCATA  | TTACATGCCA | TATGCTTCAA | TATTCGAACT | GGACACTTCA |
|      |             |            |            | PacI       |            |
|      |             |            |            | ~~~~~      |            |
| 2301 | GAAAAATGGC  | GCAGATTGTG | CGACATTTT  | TTTGTCTGCC | GTTTAATTAA |
|      | CTTTTACCG   | CGCTAACAC  | GCTGTAAAAA | AAACAGACGG | CAAATTAATT |
|      |             | FseI       |            |            |            |
|      |             | ~~~~~      |            |            |            |
| 2351 | GGGGGGGGGC  | CGGCCATTAT | CAAAAAGGAT | CTCAAGAAGA | TCCTTTGATC |
|      | CCCCCCCCCG  | GCCGGTAATA | GTTTTTCCTA | GAGTCTTCT  | AGGAAACTAG |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |            |            |             |             |             |
|------|------------|------------|-------------|-------------|-------------|
| 2401 | TTTTCTACGG | GGTCTGACGC | TCAGTGGAAC  | GAAAACTCAC  | GTTAAGGGAT  |
|      | AAAAGATGCC | CCAGACTGCG | AGTCACCTTG  | CTTTTGAGTG  | CAATTCCCTA  |
| 2451 | TTTGGTCATG | AGATTATCAA | AAAGATCTTT  | CACCTAGATC  | CTTTTAAATT  |
|      | AAACCAGTAC | TCTAATAGTT | TTTCCTAGAA  | GTGGATCTAG  | GAAAAATTAA  |
| 2501 | AAAAATGAAG | TTTTAAATCA | ATCTAAAGTA  | TATATGAGTA  | AACTTGGTCT  |
|      | TTTTTACTTC | AAAATTTAGT | TAGATTTTCAT | ATATACTCAT  | TTGAAACCAGA |
| 2551 | GACAGTTACC | CAATGCTTAA | TCAGTGAGGC  | ACCTATCTCA  | GCGATCTGTC  |
|      | CTGTCAATGG | GTTACGAATT | AGTCACTCCG  | TGGATAGAGT  | CGCTAGACAG  |
| 2601 | TATTTCTGTT | ATCCATAGTT | GCCTGACTCC  | CCGTCGTGTA  | GATAACTACG  |
|      | ATAAAGCAAG | TAGGTATCAA | CGGACTGAGG  | GGCAGCACAT  | CTATTGATGC  |
| 2651 | ATACGGGAGG | GCTTACCATC | TGGCCCCAGT  | GCTGCAATGA  | TACCGCGAGA  |
|      | TATGCCCTCC | CGAATGGTAG | ACCGGGGTCA  | CGACGTTACT  | ATGGCGCTCT  |
| 2701 | CCCACGCTCA | CCGGCTCCAG | ATTATCAGC   | AATAAACCCAG | CCAGCCGGAA  |
|      | GGTGCGGAGT | GGCCGAGGTC | TAAATAGTCG  | TTATTTGGTC  | GGTCGGCCTT  |
| 2751 | GGGCCGAGCG | CAGAAGTGGT | CCTGCAACTT  | TATCCGCCCTC | CATCCAGTCT  |
|      | CCCGGCTCGC | GTCTTCACCA | GGACGTTGAA  | ATAGGCGGAG  | GTAGGTCAGA  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |            |             |            |            |            |
|------|------------|-------------|------------|------------|------------|
| 2801 | ATTAACTGTT | GCCGGGAAGC  | TAGAGTAAGT | AGTTCGCCAG | TTAATAGTTT |
|      | TAATTGACAA | CGGCCCTTCG  | ATCTCATTCA | TCAAGCGGTC | AATTATCAAA |
| 2851 | GCGCAACGTT | GTTGCCATTG  | CTACAGGCAT | CGTGGTGTCA | CGCTCGTCGT |
|      | CGCGTTGCAA | CAACGGTAAC  | GATGTCCGTA | GCACCACAGT | GCGAGCAGCA |
| 2901 | TTGGTATGGC | TTCAATTCAGC | TCCGGTTCCC | AACGATCAAG | GCGAGTTACA |
|      | AACCATACCG | AAGTAAGTCG  | AGGCCAAGGG | TTGCTAGTTC | CGCTCAATGT |
| 2951 | TGATCCCCCA | TGTTGTGCAA  | AAAAGCGGTT | AGCTCCTTCG | GTCCTCCGAT |
|      | ACTAGGGGGT | ACAACACGTT  | TTTTCGCCAA | TCGAGGAAGC | CAGGAGGCTA |
| 3001 | CGTTGTCAGA | AGTAAGTTGG  | CCGCAGTGTT | ATCACTCATG | GTTATGGCAG |
|      | GCAACAGTCT | TCATTCAACC  | GGCGTCACAA | TAGTGAGTAC | CAATACCGTC |
| 3051 | CACTGCATAA | TTCTCTTACT  | GTCATGCCAT | CCGTAAGATG | CTTTTCTGTG |
|      | GTGACGTATT | AAGAGAATGA  | CAGTACGGTA | GGCATTCTAC | GAAAAGACAC |
| 3101 | ACTGGTGAGT | ACTCAACCAA  | GTCAATCTGA | GAATAGTGA  | TGCGGCGACC |
|      | TGACCACTCA | TGAGTTGGTT  | CAGTAAGACT | CTTATCACAT | ACGCCGCTGG |
| 3151 | GAGTTGCTCT | TGCCCGGCGT  | CAATACGGGA | TAATACCGCG | CCACATAGCA |
|      | CTCAACGAGA | ACGGCCGCGA  | GTTATGCCCT | ATTATGGCGC | GGTGATCGT  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

| XmnI  |                                                                                                                   |
|-------|-------------------------------------------------------------------------------------------------------------------|
| 3201  | GAAC TTAAA AGTGCTCATC ATTGGA AAC GTTCTTCGGG GCGAA AACTC<br>CTTGAAATTT TCACGAGTAG TAACCTTTTG CAAGAAGCCC CGCTTTTGAG |
| 3251  | TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGCGC<br>AGTTCCCTAGA ATGGCGACAA CTCTAGGTCA AGCTACATTG GGTGAGCGCG |
| 3301  | ACCCA ACTGA TCCTCAGCAT CTTT TACTTT CACCAGCGTT TCTGGGTGAG<br>TGGGTTGACT AGGAGTCGTA GAAATGAAA GTGGTCGCAA AGACCCACTC |
| 3351  | CAAAAACAGG AAGGCAAAAT GCCGCAAAA AGGGAATAAG GCGGACACGG<br>GTTTTTGTCC TTCCGTTT TA CGCGTTTTC TCCCTTATTC CCGCTGTGCC   |
| 3401  | AAATGTTGAA TACTCATACT CTTCCCTTTT CAATATTATT GAAGCATTTA<br>TTTACAACCTT ATGAGTATGA GAAGGAAAAA GTTATAATAA CTTCGTAAAT |
| BsrGI |                                                                                                                   |
| 3451  | TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAAT<br>AGTCCCAATA ACAGAGTACT CGCCTATGTA TAAACTTA                            |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

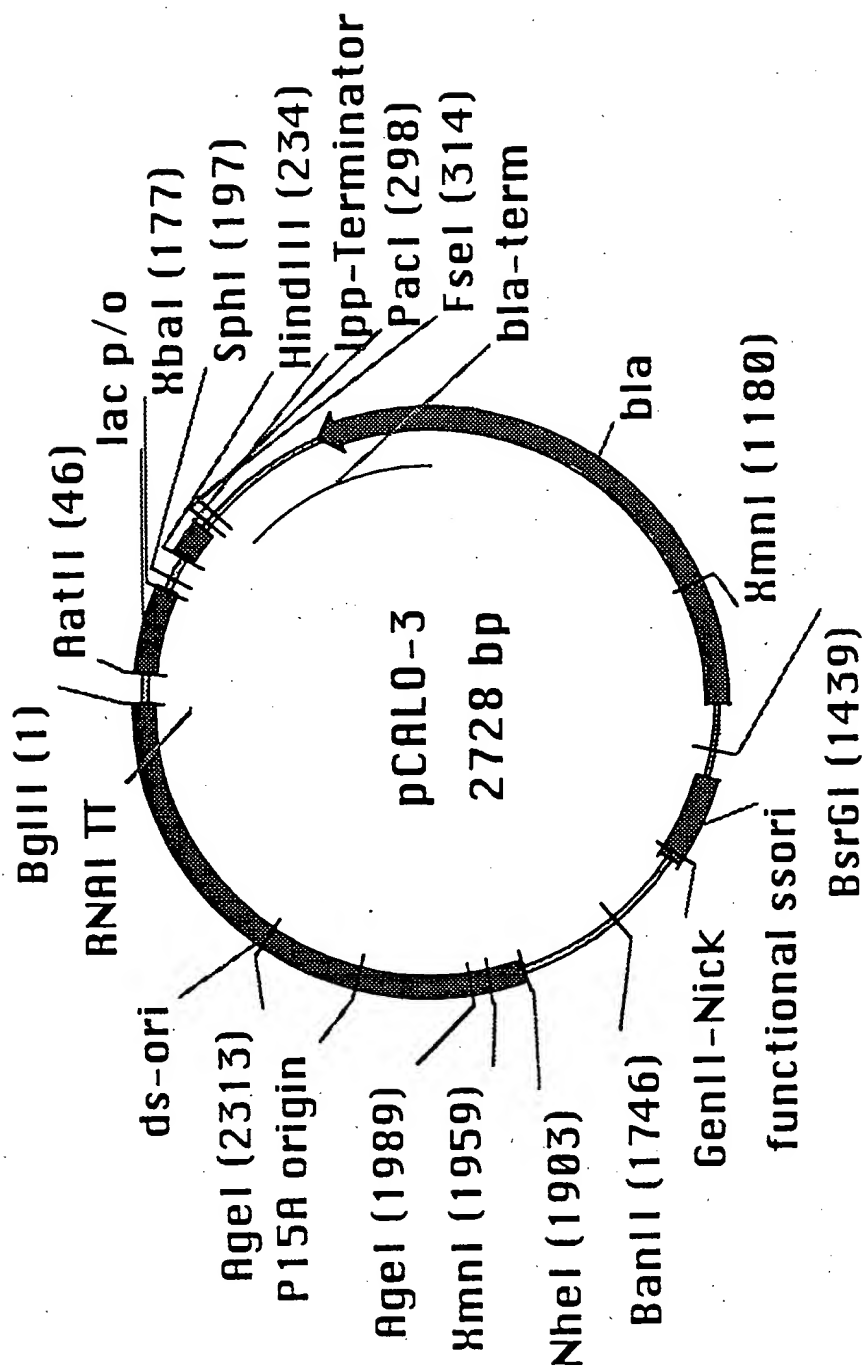


Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

| pCALO-3: |                                                         | AatII   |       |
|----------|---------------------------------------------------------|---------|-------|
| BgIII    |                                                         | ~~~~~   |       |
| 1        | GATCTCATAA CTTCTGTATAA TGTATGCTAT ACGAAGTTAT GACGTCTAAT |         |       |
|          | CTAGAGTATT GAAGCATATT ACATACGATA TGCTTCAATA CTGCAGATTA  |         |       |
| 51       | GTGAGTTAGC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC  |         |       |
|          | CACTCAATCG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG  |         |       |
| 101      | GGCTCGTATG TTGTGTGGAA TTGTGAGCCG ATAACAATT CACACAGGAA   |         |       |
|          | CCGAGCATAA AACACACCTT AACACTCGCC TATTGTTAAA GTGTGTCCCTT |         |       |
|          |                                                         | XbaI    | SphI  |
|          |                                                         | ~~~~~   | ~~~~~ |
| 151      | ACAGCTATGA CCATGATTAC GAATTTCCTAG ACCCCCCCCC CGCATGCCAT |         |       |
|          | TGTCGATACT GGTAATAATG CTTAAAGATC TGGGGGGGGG GCGTACGGTA  |         |       |
|          |                                                         | HindIII |       |
|          |                                                         | ~~~~~   |       |
| 201      | AACTTCGTAT AATGTACGCT ATACGAAGTT ATAAGCTTGA CCTGTGAAGT  |         |       |
|          | TTGAAGCATA TTACATGCCG TATGCTTCAA TATTCGAACT GGACACTTCA  |         |       |
|          |                                                         |         | PacI  |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

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251  GAAAAATGGC GCAGATTGTG CGACATTTT TTTGTCTGCC GTTTAATTAA ~~~~~
      CTTTATTACCG CGTCTAACAC GCTGTAAAAA AACAGACGG CAAATTAAAT

      FseI
      ~~~~~
301  GGGGGGGGC CGGCCATTAT CAAAAGGAT CTCAAGAAGA TCCTTTGATC
      CCCCCCCCG GCCGGTAATA GTTTTCCTA GAGTCTTCT AGGAAACTAG

351  TTTTCTACGG GGCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT
      AAAAGATGCC CCAGACTGCG AGTCACCTTG CTTTGTAGTG CAATTCCCTA

401  TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT
      AAACCAGTAC TCTAATAGTT TTTCCCTAGAA GTGGATCTAG GAAAATTTAA

451  AAAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT
      TTTTACTTC AAAATTTAGT TAGATTTCAT ATATACTCAT TTGAACCAGA

501  GACAGTTACC CAATGCTTAA TCAGTGAGGC ACCTATCTCA GCGATCTGTC
      CTGTCAATGG GTTACGGAAT AGTCACTCCG TGGATAGAGT CGCTAGACAG

551  TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG
      ATAAAGCAAG TAGGTATCAA CCGACTGAGG GGCAGCACAT CTATTGATGC

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|     |                                                         |                                                          |
|-----|---------------------------------------------------------|----------------------------------------------------------|
| 601 | ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA  | TATGCCCTCC CGAATGGTAG ACCGGGTCA CGACGTTACT ATGGCGCTCT    |
| 651 | CCCACGCTCA CCGGCTCCAG ATTATCAGC AATAAACCCAG CCAGCCGGAA  | GGGTGCGAGT GGCCGAGGTC TAAATAGTCG TTATTTGGTC GTTCGGCCCTT  |
| 701 | GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCCTC CATCCAGTCT | CCCGGCTCGC GTCTTCACCA GGACGTTGAA ATAGGCGGAG GTAGGTCAGA   |
| 751 | ATTAACTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCCAG TTAATAGTTT | TAAATTGACAA CGGCCCTTCG ATCTCATTTCA TCAAGCGGTC AATTATCAAA |
| 801 | GCGCAACGTT GTTGCCATTG CTACAGGCAT CGTGGTGTCA CGTCCGTCCGT | CGCGTTGCAA CAACGGTAAC GATGTCCGTA GCACCACAGT GCGAGCAGCA   |
| 851 | TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA  | AACCATACCG AAGTAAGTCG AGGCCAAGGG TTGCTAGTTC CGCTCAATGT   |
| 901 | TGATCCCCCA TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG GTCCCTCCGAT | ACTAGGGGGT ACAACACGTT TTTTCGCCCAA TCGAGGAAGC CAGGAGGCTA  |
| 951 | CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG  | GCAACAGTCT TCATTCAACC GCGTCAACA TAGTGAGTAC CAATACCGTC    |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |                 |                |                  |              |               |
|------|-----------------|----------------|------------------|--------------|---------------|
| 1001 | CACTGCATAA      | TTCCTCTTACT    | GTCATGCCAT       | CCGTAAGATG   | CTTTCTGTG     |
|      | GTGACGTATT      | AAGAGAAATGA    | CAGTACGGTA       | GGCATTCTAC   | GAAAAGACAC    |
| 1051 | ACTGGTGAGT      | ACTCAACCAA     | GTCATTCTGA       | GAATAGTGA    | TGCGGCGACC    |
|      | TGACCACTCA      | TGAGTTGGTT     | CAGTAAGACT       | CTTATCACAT   | ACGCCGCTGG    |
| 1101 | GAGTTGCTCT      | TGCCCGGCGT     | CAATACGGGA       | TAATACCGCG   | CCACATAGCA    |
|      | CTCAACGAGA      | ACGGGCCGCA     | GTTATGCCCT       | ATTATGGCGC   | GGTGTATCGT    |
|      |                 |                | XmnI             |              |               |
|      |                 |                | ~~~~~            |              |               |
| 1151 | GAAC TT TAA A   | AGTGCTCATC     | ATTGGA A A A C   | GTTCTTCGGG   | GCGAA A A CTC |
|      | CTTGAA A A TTT  | TCACGAGTAG     | TAACCTTTTG       | CAAGAAGCCC   | CGCTTTTGAG    |
| 1201 | TCAAGGATCT      | TACCGCTGTT     | GAGATCCAGT       | TCGATGTAAC   | CCACTCGCGC    |
|      | AGTTCCCTAGA     | ATGGCGACAA     | CTCTAGGTCA       | AGCTACATTG   | GGTGAGCGCG    |
| 1251 | ACCCA A CTGA    | TCCTCAGCAT     | CTTTTACTTT       | CACCAGCGTT   | TCTGGGTGAG    |
|      | TGGGTTGACT      | AGGAGTCGTA     | GAA A A TGAA A   | GTGGTCGCAA   | AGACCCACTC    |
| 1301 | CA A A A A CAGG | AAGC A A A A T | GCCG C A A A A A | AGGGA A TAAG | GCGACACCGG    |
|      | GTTT T TGTCC    | TTCCG G TTTTA  | CGCG G TTTT      | TCCCTTATTC   | CCGCTGTGCC    |
| 1351 | AAATGTTGAA      | TACTCATACT     | CTTCC TTTT       | CAATATTATT   | GAAGCATTTA    |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |             |             |             |             |            |
|------|-------------|-------------|-------------|-------------|------------|
|      | TTTACAACCTT | ATGAGTATGA  | GAAGGAAAAA  | GTTATAATAA  | CTTCGTAAAT |
|      |             |             |             | BsrGI       |            |
|      |             |             |             | ~~~~~       |            |
| 1401 | TCAGGGTTAT  | TGTCTCATGA  | GCGGATACAT  | ATTTGAATGT  | ACATGAAATT |
|      | AGTCCCAATA  | ACAGAGTACT  | CGCCTATGTA  | TAAACTTACA  | TGTACTTTAA |
| 1451 | GTA AACGTTA | ATATTTTGT   | AAAATTCGCG  | TTAAATTTT   | GTAAATCAG  |
|      | CATTTGCAAT  | TATAAAACAA  | TTTTAAGCGC  | AATTAAAAA   | CAATTTAGTC |
| 1501 | CTCATTTT    | AACCAATAGG  | CCGAAATCGG  | CAAAATCCCT  | TATAAATCAA |
|      | GAGTAAAAAA  | TTGGTTATCC  | GGCTTTAGCC  | GTTTtaggga  | ATATTTAGTT |
| 1551 | AAGAATAGAC  | CGAGATAGG   | TTGAGTGTG   | TTCCAGTTTG  | GAACAAGAGT |
|      | TTCTTATCTG  | GCTCTATCCC  | AACTCACAAAC | AAGTCAAAC   | CTTGTTCTCA |
| 1601 | CCACTATTAA  | AGAACGTGGA  | CTCCAACGTC  | AAAGGGCGAA  | AAACCGTCTA |
|      | GGTGATAAAT  | TCTTGACACCT | GAGGTTGCAG  | TTTCCCCGCTT | TTTGGCAGAT |
| 1651 | TCAGGGCGAT  | GGCCCACTAC  | GAGAACCATC  | ACCCTAATCA  | AGTTTTTGG  |
|      | AGTCCCGCTA  | CCGGGTGATG  | CTCTTGGTAG  | TGGGATTAGT  | TCAAAAAACC |
|      |             |             |             | BanII       |            |
|      |             |             |             | ~~~~~       |            |

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Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|      |            |             |            |             |             |
|------|------------|-------------|------------|-------------|-------------|
| 1701 | GGTCGAGGTG | CCGTAAAGCA  | CTAAATCGGA | ACCTAAAGG   | GAGCCCCCGA  |
|      | CCAGCTCCAC | GGCATTTTCGT | GATTAGCCT  | TGGATTTC    | CTCGGGGGCT  |
| 1751 | TTTAGAGCTT | GACGGGAAA   | GCCGGCGAAC | GTGGCGAGAA  | AGGAAGGGAA  |
|      | AAATCTCGAA | CTGCCCTTT   | CGGCCGCTTG | CACCGCTCTT  | TCCCTTCCCTT |
| 1801 | GAAAGCGAAA | GGAGCGGGCG  | CTAGGGCGCT | GGCAAGTGA   | GCGGTCACGC  |
|      | CTTTCGCTTT | CCTCGCCCGC  | GATCCCGCGA | CCGTTACAT   | CGCCAGTGCG  |
| 1851 | TGCGCGTAAC | CACCACACCC  | GCCGCGCTTA | ATGCGCCGCT  | ACAGGGCGCG  |
|      | ACCGGCATTG | GTGGTGTGGG  | CGCGCGGAAT | TACGCGGCGA  | TGTCCCCGCGC |
|      | NheI       |             |            |             |             |
|      | ~~~~~      |             |            |             |             |
| 1901 | TGCTAGCGGA | GTGTATACTG  | GCTTACTATG | TTGGCACTGA  | TGAGGGTGTC  |
|      | ACGATCGCCT | CACATATGAC  | CGAATGATAC | AACCGTGACT  | ACTCCCACAG  |
|      | XmnI       |             |            |             |             |
|      | ~~~~~      |             |            |             |             |
| 1951 | AGTGAAGTGC | TTCATGTGGC  | AGGAGAAAAA | AGGCTGCACC  | GGTGCGTCAG  |
|      | TCACTTCACG | AAGTACACCG  | TCCTCTTTT  | TCCGACGTGG  | CCACGCAGTC  |
| 2001 | CAGAATATGT | GATACAGGAT  | ATATTCCGCT | TCCCTCGCTCA | CTGACTCGCT  |
|      | GTCTTATACA | CTATGTCCTA  | TATAAGCGCA | AGGAGCGAGT  | GACTGAGCGA  |
|      | AgeI       |             |            |             |             |
|      | ~~~~~      |             |            |             |             |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|               |             |            |            |             |             |
|---------------|-------------|------------|------------|-------------|-------------|
| 2051          | ACGCTCGGTC  | GTTCGACTGC | GGCGAGCCGA | AATGGCTTAC  | GAACGGGGCG  |
|               | TGCGAGCCAG  | CAAGCTGACG | CCGCTCGCCT | TTACCGAATG  | CTTGCCCCCG  |
| 2101          | GAGATTTCCT  | GGAAGATGCC | AGGAAGATAC | TTAACAGGGA  | AGTGAGAGGG  |
|               | CTCTAAAGGA  | CCTTCTACGG | TCCTTCTATG | AATTGTCCCT  | TCACTCTCCC  |
| 2151          | CCGCGGCAAA  | GCCGTTTTC  | CATAGGCTCC | GCCCCCCTGA  | CAAGCATCAC  |
|               | GGCGCCGTTT  | CGGCAAAAG  | GTATCCGAGG | CGGGGGGACT  | GTTCGTAGTG  |
| 2201          | GAAATCTGAC  | GCTCAAATCA | GTGGTGGCGA | AACCCGACAG  | GACTATAAAG  |
|               | CTTTAGACTG  | CGAGTTTAGT | CACCACCGCT | TTGGGCTGTC  | CTGATATTTC  |
| 2251          | ATACCAGGCG  | TTTCCCCCTG | GCGGCTCCCT | CCTGCCGCTCT | CCTGTTCCCTG |
|               | TATGGTCCGC  | AAAGGGGGAC | CGCCGAGGGA | GGACGCGAGA  | GGACAAGGAC  |
| AgeI<br>~~~~~ |             |            |            |             |             |
| 2301          | CCTTTCGGTT  | TACCGGTGTC | ATTCCGCTGT | TATGGCCGCG  | TTTGTCTCAT  |
|               | GGAAAGCCAA  | ATGGCCACAG | TAAGGCGACA | ATACCGGCGC  | AAACAGAGTA  |
| 2351          | TCCACGCCCTG | ACACTCAGTT | CCGGGTAGGC | AGTTCGCTCC  | AAGCTGGACT  |
|               | AGTGCGGGAC  | TGTGAGTCAA | GGCCCATCCG | TCAAGCGAGG  | TTCGACCTGA  |

Figure 35a: Functional maps and sequences of additional pCAL vector modules and pCAL vectors (continued)

|       |            |            |            |             |             |
|-------|------------|------------|------------|-------------|-------------|
| 2401  | GTATGCACGA | ACCCCCCGTT | CAGTCCGACC | GCTGCCCTT   | ATCCGGTAAC  |
|       | CATACGTGCT | TGGGGGGCAA | GTCAGGCTGG | CGACGCGGAA  | TAGGCCATTG  |
| 2451  | TATCGTCTTG | AGTCCAACCC | GGAAAGACAT | GCAAAAGCAC  | CACTGGCAGC  |
|       | ATAGCAGAAC | TCAGGTTGGG | CCTTCTCTGA | CGTTTTCGTG  | GTGACCGTCG  |
| 2501  | AGCCACTGGT | AATTGATTTA | GAGGAGTTAG | TCTTGAAGTC  | ATGCGCCCGGT |
|       | TCGGTGACCA | TTAACATAAT | CTCCTCAATC | AGAACTTCAG  | TACGCGGCCA  |
| 2551  | TAAGGCTAAA | CTGAAAGGAC | AAGTTTTAGT | GACTGCCGCTC | CTCCAAGCCA  |
|       | ATTCCGATT  | GACTTTCCTG | TTCAAAATCA | CTGACGCGAG  | GAGGTTCCGT  |
| 2601  | GTTACCTCGG | TTCAAAGAGT | TGGTAGCTCA | GAGAACCTAC  | GAAAAACCGC  |
|       | CAATGGAGCC | AAGTTTCTCA | ACCATCGAGT | CTCTTGGATG  | CTTTTGGCG   |
| 2651  | CCTGCAAGGC | GGTTTTTTCG | TTTTCAGAGC | AAGAGATTAC  | GCGCAGACCA  |
|       | GGACGTTCCG | CCAAAAAAGC | AAAAGTCTCG | TTCTCTAATG  | CGCGTCTGGT  |
| BglII |            |            |            |             |             |
| 2701  | AAACGATCTC | AAGAAGATCA | TCTTATTA   |             |             |
|       | TTTGCTAGAG | TTCTTCTAGT | AGAATAAT   |             |             |

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Figure 35b: List of oligonucleotides used for synthesis of modules

M1: PCR using template

NoVspAatII: TAGACGTC

M2: synthesis

BloxA-A: TATGAGATCTCATAACTTCGTATAATGTACGCTATACG-  
AAGTTAT

BloxA-B: TAATAACTTCGTATAGCATACATTATACGAAGTTATG-  
AGATCTCA

M3: PCR, NoVspAatII as second oligo

XloxS-muta: CATTTTTGCCCTCGTTATCTACGCATGCGATAACTTCGTA-  
TAGCGTACATTATACGAAGTTATTCTAGACATGGTCATAGCTGTTTCCTG

M7-I: PCR

gIIINEW-fow: GGGGGGAATTCGGTGGTGGTGGATCTGCGTGCGCTG-  
AAACGGTTGAAAGTTG

gIIINEW-rev: CCCCCCAAGCTTATCAAGACTCCTTATTACG

M7-II: PCR

gIIIss-fow: GGGGGGGGAATTCGGAGGCGGTTCCGGTGGTGGC

M7-III: PCR

gIIIsupernew-fow: GGGGGGGGAATTCGAGCAGAAGCTGATCTCT-  
GAGGAGGATCTGTAGGGTGGTGGCTCTGGTTCCGGTGATTTG

Figure 35b: List of oligonucleotides used for synthesis of modules (continued)

M8: synthesis

lox514-A: CCATAACTTCGTATAATGTACGCTATACGAAGTTATA

lox514-B: AGCTTATAACTTCGTATAGCGTACATTATACGAAGT-  
TATGGCATG

M9II: synthesis

M9II-fow: AGCTTGACCTGTGAAGTGAAAAATGGCGCAGATT-  
GTGCGACATTTTTTTTGTCTGCCGTTTAATTAAAGGGGGGGT

M9II-rev: GTACACCCCCCCCCAGGCCGGCCCCCCCCCCCCCTTTAA-  
TTAAACGGCAGACAAAAAAAATGTCGCACAATCTGCG

M10II: assembly PCR with template

bla-fow: GGGGGGGTGTACATTCAAATATGTATCCGCTCATG

bla-seq4: GGGTTACATCGAACTGGATCTC

bla1-muta: CCAGTTCGATGTAACCCACTCGCGCACCCAACTGATC-  
CTCAGCATCTTTTACTTTCACC

bla1-muta: ACTCTAGCTTCCCGGCAACAGTTAATAGACTGGATG-  
GAGGCGG

bla-NEW: CTGTTGCCGGGAAGCTAGAGTAAG

bla-rev: CCCCCCTTAATTAAGGGGGGGGGCCGGCCATTATCAAA-  
AAGGATCTCAAGAAGATCC

M11II/III: PCR, site-directed mutagenesis

Figure 35b: List of oligonucleotides used for synthesis of modules (continued)

f1-fow: GGGGGGGGCTAGCACGCGCCCTGTAGCGGCGCATTA

f1-rev: CCCCCCTGTACATGAAATTGTAAACGTTAATATTTTG

f1-t133.muta: GGGCGATGGCCCACTACGAGAACCATCACCTAATC

M12: assembly PCR using template

p15-fow: GGGGGGAGATCTAATAAGATGATCTTCTTGAG

p15-NEWI: GAGTTGGTAGCTCAGAGAACCTACGAAAAACCGCCCTG-  
CAAGGCG

p15-NEWII: GTAGGTTCTCTGAGCTACCAACTC

p15-NEWIII: GTTCCCCCTGGCGGCTCCCTCCTGCGCTCTCCTGTTCT-  
GCC

p15-NEWIV: AGGAGGGAGCCGCCAGGGGGAAAC

p15-rev: GACATCAGCGCTAGCGGAGTGTATAC

M13: synthesisBloxB-A: GATCTCATAACTTCGTATAATGTATGCTATACGAAGTTA-  
TTCABloxB-B: GATCTGAATAACTTCGTATAGCATACATTATACGAAGTTA-  
TGAGAM14-Ext2: PCR, site-directed mutagenesis

ColEXT2-fow: GGGGGGGGAGATCTGACCAAATCCCTTAACGTGAG

Col-mutal: GGTATCTGCGCTCTGCTGTAGCCAGTTACCTTCGG

Figure 35b: List of oligonucleotides used for synthesis of modules (continued)

Col-rev: CCCCCCGCTAGCCATGTGAGCAAAAGGCCAGCAA

M17: assembly PCR using template

CAT-1: GGGACGTCGGGTGAGGTTCCAAC

CAT-2: CCATACGGAACTCCGGGTGAGCATTTCATC

CAT-3: CCGGAGTTCGTATGG

CAT-4: ACGTTTAAATCAAACTGG

CAT-5: CCAGTTTTGATTTAAACGTAGCCAATATGGACAACCTTCTTC-

GCCCCCGTTTTCACTATGGGCAAATATT

CAT-6: GGAAGATCTAGCACCAGGCGTTTAAG

M41: assembly PCR using template

LAC1: GAGGCCGGCCATCGAATGGCGCAAAAC

LAC2: CGCGTACCGTCCTCATGGGAGAAAATAATAC

LAC3: CCATGAGGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCA-  
TTGGGTCACCAGCAAATCCGCTGTTAGCTGGCCCATTAAG

LAC4: GTCAGCGGCGGGATATAACATGAGCTGTCCTCGGTATCGTCG

LAC5: GTTATATCCCGCCGCTGACCACCATCAAAC

LAC6: CATCAGTGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGT4TTG-  
GGAGCCAGGGTGGTTTTTC

LAC7: GGTTAATTAACCTCACTGCCCCGCTTCCAGTCGGGAAACCTGTCGTGCC-  
AGCTGCATCAGTGAATCGGCCAAC

M41-MCS-fow: CTAGACTAGTGTTTAAACCGGACCGGGGGGGGGGCTT-  
AAGGGGGGGGGGGG

Figure 35b: List of oligonucleotides used for synthesis of modules (continued)

M41-MCS-rev: CTAGCCCCCCCCCCCCCTTAAGCCCCCCCCCGGTCCGGT-  
TTAAACACTAGT

M41-fow: CTAGACTAGTGTTTAAACCGGACCGGGGGGGGGGCTTAA-  
GGGGGGGGGGGGG

M41-rev: CCCCCCTTAAGTGGGCTGCAAAACAAAACGGCCTCC-  
TGTCAGGAAGCCGCTTTTATCGGGTAGCCTCACTGCCCCGCTTCC

M41-A2: GTTGTTGTGCCACGCGGTAGGAATGTAATTCAGCTCCGC

M41-B1: AACCGCGTGGCACAACAAC

M41-B2: CTCGTTCTACCATCGACACGACCACGCTGGCACCCAGTTG

M41-C1: GTGTCGATGGTAGAACGAAG

M41-CII: CCACAGCAATAGCATCCTGGTCATCCAGCGGATAGTT-  
AATAATCAGCCCACTGACACGTTGCGCGAG

M41-DI: GACCAGGATGCTATTGCTGTGG

M41-DII: CAGCGCGATTGCTGGTGGCCCAATGCGACCAGATGC

M41-EI: CACCAGCAAATCGCGCTG

M41-EII: CCCGGACTCGGTAATGGCACGCATTGCGCCCAGCGCC

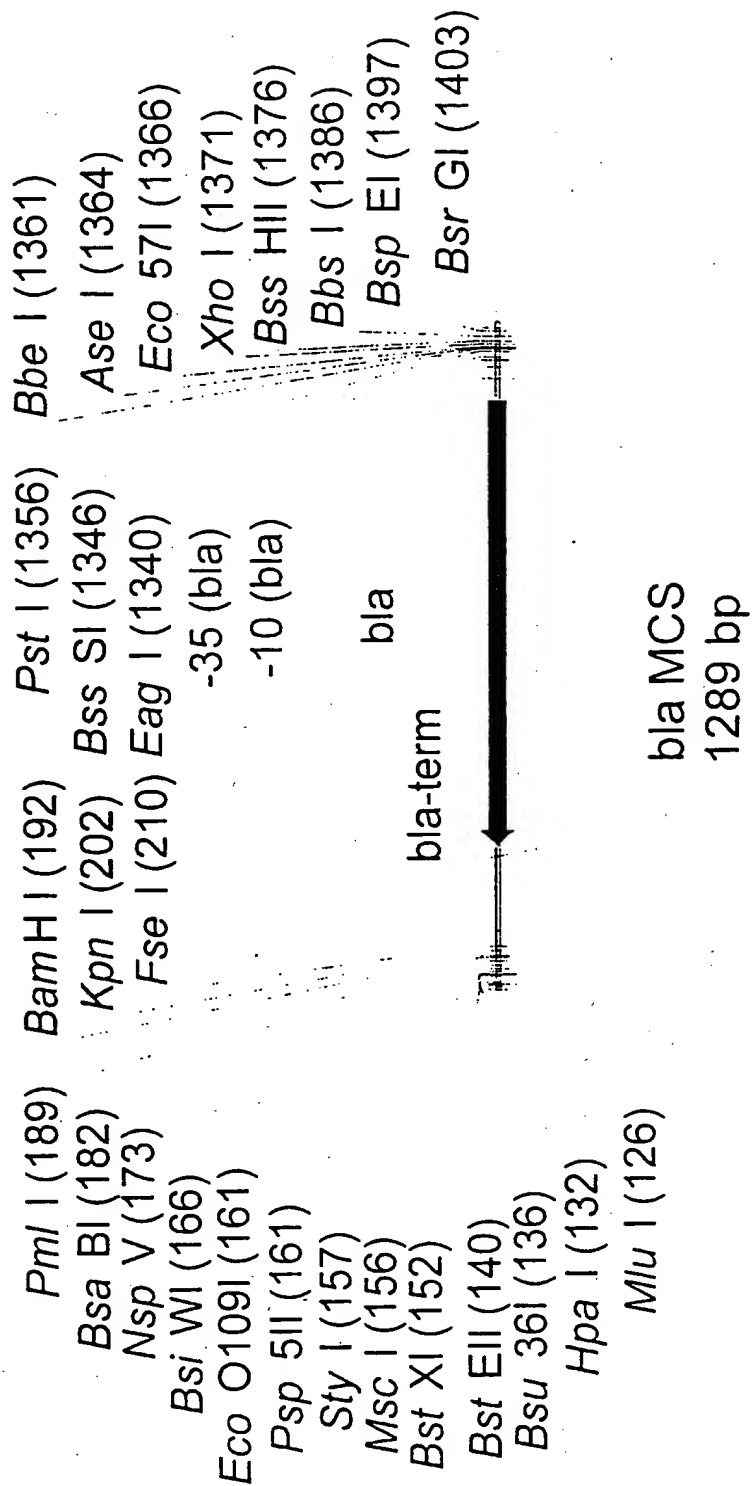
M41-FI: GCCATTACCGAGTCCGGG

M42: synthesis

Eco-H5-Hind-fow: AATCCACCATCATCACCATTGACGTCTA

Eco-H5-Hind-rev: AGCTTAGACGTCAATGGTGATGATGGTGG



Figure 36: functional map and sequence of  $\beta$ -lactamase-MCS module

|     | Styl       |             |             |            |              |       |       |       |       |       |
|-----|------------|-------------|-------------|------------|--------------|-------|-------|-------|-------|-------|
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
|     | Psp5II     |             |             |            |              |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
|     | EcoO109I   |             |             |            |              |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
|     | MluI       | Bsu36I      | BstXI       |            |              | MscI  |       |       | BsiWI | NspV  |
|     | ~~~~~      | ~~~~~       | ~~~~~       | ~~~~~      | ~~~~~        | ~~~~~ | ~~~~~ | ~~~~~ | ~~~~~ | ~~~~~ |
|     | HpaI       | BstEII      |             |            |              |       |       |       |       |       |
|     | ~~~~~      | ~~~~~       |             |            |              |       |       |       |       |       |
| 126 | CGCGTTAAC  | TCAGGTGACC  | AAGCCCCCTGG | CCAAGGTCCC | GTACGTTCCA   |       |       |       |       |       |
|     | GCACAATTGG | AGTCCACTGG  | TTCGGGGACC  | GGTCCAGGG  | CATGCAAGCT   |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
|     | PmlI       |             |             |            |              |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
|     | NspVBsaBI  | BamHI       | KpnI        |            |              | FseI  |       |       |       |       |
|     | ~~~~~      | ~~~~~       | ~~~~~       | ~~~~~      | ~~~~~        | ~~~~~ |       |       |       |       |
| 176 | AGATTACCAT | CACGTGGATC  | CGGTACCAGG  | CCGGCCATTA | TCAAAAAGGA   |       |       |       |       |       |
|     | TCTAATGGTA | GTGCACCTAG  | GCCATGGTCC  | GGCCGGTAAT | AGTTTTCCT    |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
| 226 | TCTCAAGAAG | ATCCTTTTGAT | CTTTTCTACG  | GGGTCTGACG | CTCAGTGGAA   |       |       |       |       |       |
|     | AGAGTTCTTC | TAGGAAACTA  | GAAAAGATGC  | CCCAGACTGC | GAGTCACCTT   |       |       |       |       |       |
|     | ~~~~~      |             |             |            |              |       |       |       |       |       |
| 276 | CGAAAACTCA | CGTTAAGGGA  | TTTTTGGTCAT | GAGATTATCA | AAAAGGATCT   |       |       |       |       |       |
|     | GCTTTTGAGT | GCAATTCCCT  | AAAACCCAGTA | CTCTAATAGT | TTTTTCCCTAGA |       |       |       |       |       |

Figure 36: functional map and sequence of  $\beta$ -lactamase-MCS module (continued)

|     |             |             |            |             |             |
|-----|-------------|-------------|------------|-------------|-------------|
| 326 | TCACCTAGAT  | CCTTTTAAAT  | TAAAAATGAA | GTTTAAATC   | AATCTAAAGT  |
|     | AGTGGATCTA  | GGAAAAATTA  | ATTTTACTT  | CAAAATTTAG  | TTAGATTTC   |
| 376 | ATATATGAGT  | AAACTTGGTC  | TGACAGTTAC | CAATGCTTAA  | TCAGTGAGGC  |
|     | TATATACTCA  | TTTGAACCCAG | ACTGTCAATG | GTTACGAATT  | AGTCACTCCG  |
| 426 | ACCTATCTCA  | CGATCTGTC   | TATTTCGTTT | ATCCATAGTT  | GCCTGACTCC  |
|     | TGGATAGAGT  | CGCTAGACAG  | ATAAGCAAG  | TAGGTATCAA  | CGGACTGAGG  |
| 476 | CCGTCGTGTA  | GATAACTACG  | ATACGGGAGG | GCTTACCATC  | TGGCCCCCAGT |
|     | GGCAGCACAT  | CTATTGATGC  | TATGCCCTCC | CGAATGGTAG  | ACCGGGGTCA  |
| 526 | GCTGCAATGA  | TACCGCGAGA  | CCCACGCTCA | CCGGCTCCAG  | ATTATCAGC   |
|     | CGACGTTACT  | ATGGCGCTCT  | GGTGCGAGT  | GGCCGAGGTC  | TAAATAGTCG  |
| 576 | AATAAACCCAG | CCAGCCGGAA  | GGGCCGAGCG | CAGAAAGTGGT | CCTGCAACTT  |
|     | TTATTTGGTC  | GGTCGGCCTT  | CCCGGCTCGC | GTCCTCACCA  | GGACGTTGAA  |
| 626 | TATCCGCCCTC | CATCCAGTCT  | ATTAAGTGT  | GCCGGGAAGC  | TAGAGTAAGT  |
|     | ATAGGCGGAG  | GTAGTTCAGA  | TAAATGACAA | CGGCCCTTCG  | ATCTCATTTCA |
| 676 | AGTTCGCCAG  | TTAATAGTTT  | GCGCAACGTT | GTGCCCATTG  | CTACAGGCAT  |
|     | TCAAGCGGTC  | AATTATCAAA  | CGCGTTGCAA | CAACGGTAAC  | GATGTCCGTA  |

Figure 36: functional map and sequence of  $\beta$ -lactamase-MCS module (continued)

|      |             |            |            |             |             |
|------|-------------|------------|------------|-------------|-------------|
| 726  | CGTGGTGTCA  | CGCTCGTCGT | TTGGTATGGC | TTCAATTCAGC | TCCGGTTCCC  |
|      | GCACCCACAGT | GCGAGCAGCA | AACCATACCG | AAGTAAGTCG  | AGGCCAAGGG  |
| 776  | AACGATCAAG  | GCGAGTTACA | TGATCCCCCA | TGTTGTGCAA  | AAAAGCGGTT  |
|      | TTGCTAGTTC  | CGCTCAATGT | ACTAGGGGGT | ACAACACGTT  | TTTTCGCCAA  |
| 826  | AGCTCCTTCG  | GTCCTCCGAT | CGTTGTCAGA | AGTAAGTTGG  | CCGCAGTGTT  |
|      | TCGAGGAAGC  | CAGGAGGCTA | GCAACAGTCT | TCATTC AAC  | GGCGTCACAA  |
| 876  | ATCACTCATG  | GTTATGGCAG | CACTGCATAA | TTCTCTTACT  | GTCATGCCAT  |
|      | TAGTGAGTAC  | CAATACCGTC | GTGACGTATT | AAGAGAATGA  | CAGTACGGTA  |
| 926  | CCGTAAGATG  | CTTTTCTGTG | ACTGGTGAGT | ACTCAACCAA  | GTCATTCTGA  |
|      | GGCATTTCTAC | GAAAAGACAC | TGACCACTCA | TGAGTTGGTT  | CAGTAAGACT  |
| 976  | GAATAGTGTA  | TGCGGCGACC | GAGTTGCTCT | TGCCCCGGCGT | CAATACGGGA  |
|      | CTTATCACAT  | ACGCCGCTGG | CTCAACGAGA | ACGGGCCGCA  | GTTATGCCCT  |
| 1026 | TAATACCGCG  | CCACATAGCA | GAAC TTAAA | AGTGCTCATC  | ATTGGA AAAC |
|      | ATTATGGCGC  | GGTGATCGT  | CTTGAAATTT | TCACGAGTAG  | TAACCTTTTG  |
| 1076 | GTTCTTCGGG  | GCGAAAATC  | TCAAGGATCT | TACCGCTGTT  | GAGATCCAGT  |
|      | CAAGAAGCCC  | CGCTTTTGAG | AGTTCCTAGA | ATGGCGACAA  | CTCTAGGTCA  |

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11126 TCGATGTAA CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTACTTT  
AGCTACATTG GTGAGCACG TGGTTGACT AGAAGTCGTA GAAATGAAA

ISSUE

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11176 CACCAGCGTT TCTGGTGAG CAAAACACAG AAGCAAAAT GCCGCAAAA  
GTGGTCGCAA AGACCCACTC GTTTTGTCC TTCCGTTTA CGGCGTTT

11226 AGGGAATAAG GCGACACGG AAATGTTGAA TACTCATCT CTTCCTTTT  
TCCCTTATTC CCGCTGTGCC TTACAACTT ATGAGTATGA GAAGGAAAAA

11276 CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT  
GTTATAATAA CTTCGTAAAT AGTCCCAATA ACAGAGTACT CGCCTATGTA

PstI XhoI

|       |       |       |       |        |
|-------|-------|-------|-------|--------|
| EagI  | BssSI | BbeI  | AseI  | BssHII |
| ~~~~~ | ~~~~~ | ~~~~~ | ~~~~~ | ~~~~~  |

11326 ATTTGAATGT ACTCGGCCGC ACGAGCTGCA GCGCCATTA ATGGCTCGAG  
TAAACTTACA TGAGCCGGCG TGCTCGACGT CCGCGGTAAT TACCGAGCTC

```
BSSHII      BspEI BsrGI
```

Figure 36: functional map and sequence of  $\beta$ -lactamase-MCS module (continued)

|      |            |            |            |           |
|------|------------|------------|------------|-----------|
| 1376 | CGCGCTTCAG | CGCTTTGTCT | TCCGGATGTA | CATGAAATT |
|      | GCGCGAAGTC | GCGAACAGA  | AGGCCTACAT | GTACTTTAA |
|      | ECO57I     | BbsI       |            |           |
|      | ~~~~~      | ~~~~~      |            |           |

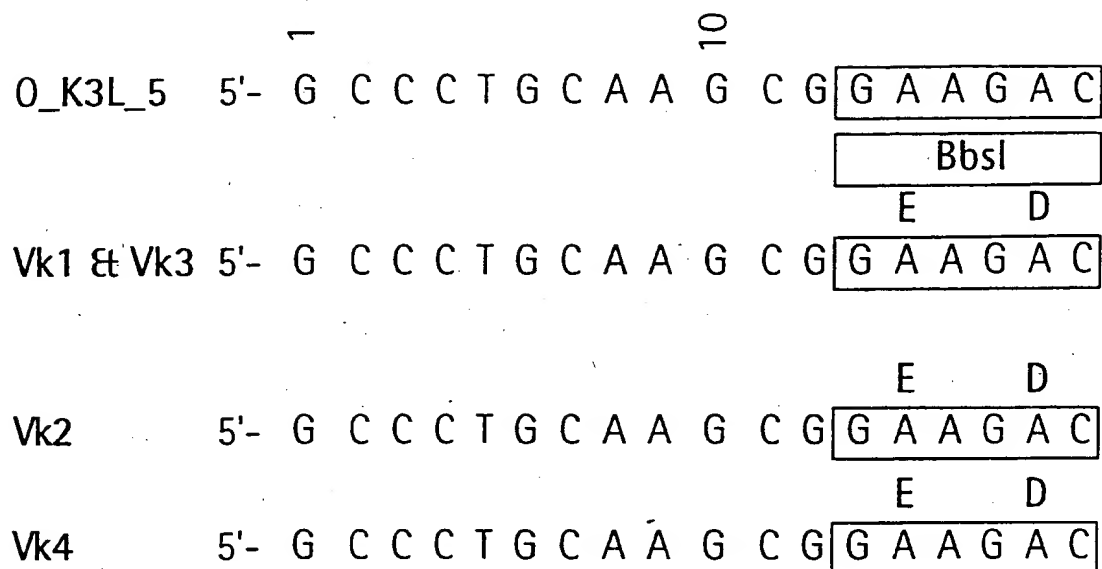
Figure 37: Oligo and primer design for V $\kappa$  CDR3 libraries





Figure 37: Oligo and primer design for V<sub>κ</sub> CDR3 libraries

|       |       |       |           |       |
|-------|-------|-------|-----------|-------|
|       | 50    |       | 60        |       |
|       |       |       | 3'- G G A |       |
| G     |       |       | T         |       |
|       |       |       | A C C T   |       |
| G     |       |       | T         |       |
|       |       |       | A C C T   |       |
| G     |       |       | T         |       |
|       |       |       | A C C T   |       |
| G C T |       |       | G C T     |       |
|       |       |       |           |       |
| G A T | G A T | G A T | G A T     | G A T |
| G A G |       |       | G A G     | G A G |
| T T T |       |       | T T T     | T T T |
| G G T | G G T | G G T | G G T     | G G T |
| C A T |       |       | C A T     | C A T |
| A T T |       |       | A T T     | A T T |
| A A G |       |       | A A G     | A A G |
| C T T |       |       | C T T     | C T T |
| A T G |       |       | A T G     | A T G |
| A A T | A A T | A A T | A A T     | A A T |
|       |       |       | C C T     | C C T |
| C A G |       |       | C A G     | C A G |
| C G T |       |       | C G T     | C G T |
| T C T | T C T | T C T | T C T     | T C T |
| A C T |       |       | A C T     | A C T |
| G T T |       |       | G T T     | G T T |
| T G G |       |       | T G G     | T G G |
| T A T | T A T |       | T A T     | T A T |
| 50% Y |       |       | 80% P     |       |

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Figure 38: Oligo and primer design for V $\lambda$  CDR3 libraries

|   |    |    |    |     |   |    |   |     |   |   |   |    |   |   |   |   |   |   |
|---|----|----|----|-----|---|----|---|-----|---|---|---|----|---|---|---|---|---|---|
|   |    | 60 |    | 70  |   | 80 |   |     |   |   |   |    |   |   |   |   |   |   |
|   |    |    |    | G   | G | G  | T | K   | L |   |   |    |   |   |   |   |   |   |
|   |    |    |    | G   | G | C  | G | G   | C | A | C | G  | A | A | G | T | T | A |
| - | G  | C  | T  | gap | G | C  | T | gap | G | C | T | G  | C | T |   |   |   |   |
|   | G  | A  | T  | G   | A | T  | G | A   | T | G | A | T  | G | A | T |   |   |   |
|   | G  | A  | G  | G   | A | G  | G | A   | G | G | A | G  | G | A | G |   |   |   |
|   | T  | T  | T  | T   | T | T  | T | T   | T | T | T | T  | T | T |   |   |   |   |
|   | G  | G  | T  | G   | G | T  | G | G   | T | G | G | T  | G | G | T |   |   |   |
|   | C  | A  | T  | C   | A | T  | C | A   | T | C | A | T  | C | A | T |   |   |   |
|   | A  | T  | T  | A   | T | T  | A | T   | T | A | T | T  | A | T | T |   |   |   |
|   | A  | A  | G  | A   | A | G  | A | A   | G | A | A | G  | A | A | G |   |   |   |
|   | C  | T  | T  | C   | T | T  | C | T   | T | C | T | T  | C | T | T |   |   |   |
|   | A  | T  | G  | A   | T | G  | A | T   | G | A | T | G  | A | T | G |   |   |   |
|   | A  | A  | T  | A   | A | T  | A | A   | T | A | A | T  | A | A | T |   |   |   |
|   | C  | C  | T  | C   | C | T  | C | C   | T | C | C | T  | C | C | T |   |   |   |
|   | C  | A  | G  | C   | A | G  | C | A   | G | C | A | G  | C | A | G |   |   |   |
|   | C  | G  | T  | C   | G | T  | C | G   | T | C | G | T  | C | G | T |   |   |   |
|   | T  | C  | T  | T   | C | T  | T | C   | T | T | C | T  | T | C | T |   |   |   |
|   | A  | C  | T  | A   | C | T  | A | C   | T | A | C | T  | A | C | T |   |   |   |
|   | G  | T  | T  | G   | T | T  | G | T   | T | G | T | T  | G | T | T |   |   |   |
|   |    |    |    |     |   |    |   |     |   |   |   | T  | G | G |   |   |   |   |
|   | T  | A  | T  | T   | A | T  | T | A   | T | T | A | T  | T | A | T |   |   |   |
|   | 18 |    |    |     |   |    |   |     |   |   |   | 19 |   |   |   |   |   |   |
|   | 18 | 18 |    |     |   |    |   |     |   |   |   | 19 |   |   |   |   |   |   |
|   | 18 | 18 | 18 |     |   |    |   |     |   |   |   | 19 |   |   |   |   |   |   |
|   |    |    |    |     |   |    |   |     |   |   |   |    |   |   |   |   |   |   |

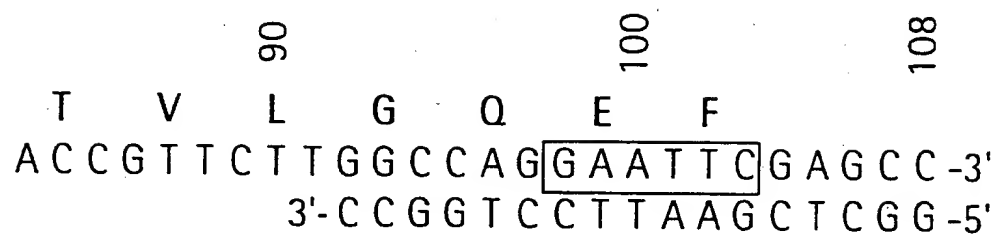
Variability

3.32E+05

5.98E+06

1.08E+08

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Figure 38: Oligo and primer design for V $\lambda$  CDR3 libraries

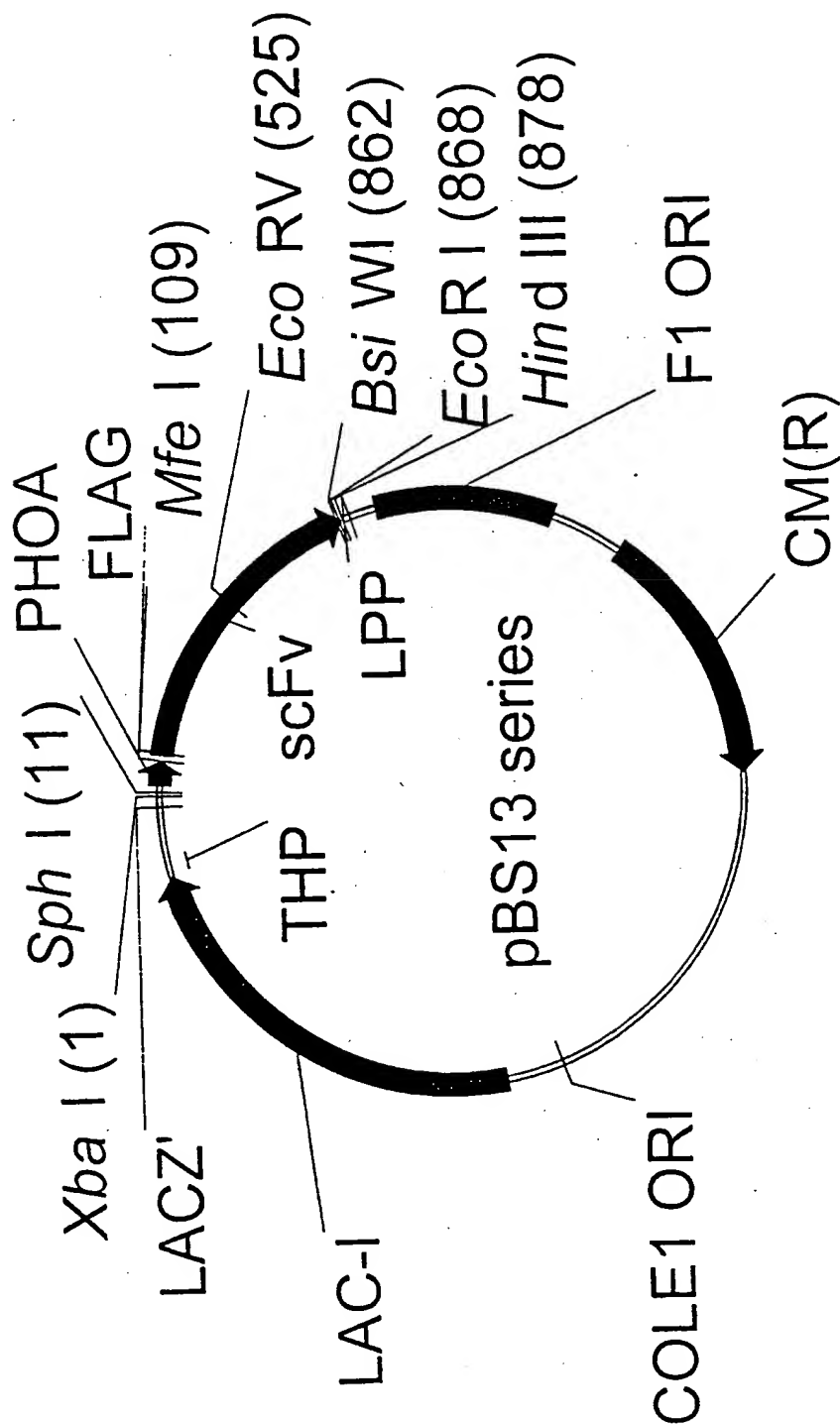


Figure 39: functional map of expression vector series pBS13

Figure 40: Expression data for HuCAL scFvs (pBS13, 30°C)

| % soluble | $\kappa 1$ | $\kappa 2$ | $\kappa 3$ | $\kappa 4$ | $\lambda 1$ | $\lambda 2$ | $\lambda 3$ |
|-----------|------------|------------|------------|------------|-------------|-------------|-------------|
| H1A       | 61%        | 58%        | 52%        | 42%        | 90%         | 61%         | 60%         |
| H1B       | 39%        | 48%        | 66%        | 48%        | 47%         | 39%         | 36%         |
| H2        | 47%        | 57%        | 46%        | 49%        | 37%         | 36%         | 45%         |
| H3        | 85%        | 67%        | 76%        | 61%        | 80%         | 71%         | 83%         |
| H4        | 69%        | 52%        | 51%        | 44%        | 45%         | 33%         | 42%         |
| H5        | 49%        | 49%        | 46%        | 67%        | 54%         | 46%         | 47%         |
| H6        | 90%        | 58%        | 54%        | 47%        | 45%         | 50%         | 51%         |

| Total amount<br>compared to H3 $\kappa 2$ | $\kappa 1$ | $\kappa 2$ | $\kappa 3$ | $\kappa 4$ | $\lambda 1$ | $\lambda 2$ | $\lambda 3$ |
|-------------------------------------------|------------|------------|------------|------------|-------------|-------------|-------------|
| H1A                                       | 289%       | 94%        | 166%       | 272%       | 20%         | 150%        | 78%         |
| H1B                                       | 219%       | 122%       | 89%        | 139%       | 117%        | 158%        | 101%        |
| H2                                        | 186%       | 223%       | 208%       | 182%       | 126%        | 60%         | 97%         |
| H3                                        | 50%        |            | 71%        | 54%        | 59%         | 130%        | 47%         |
| H4                                        | 37%        | 55%        | 60%        | 77%        | 195%        | 107%        | 251%        |
| H5                                        | 98%        | 201%       | 167%       | 83%        | 93%         | 128%        | 115%        |
| H6                                        | 65%        | 117%       | 89%        | 109%       | 299%        | 215%        | 278%        |



Figure 40: Expression data for HuCAL scFvs (pBS13, 30°C)

| Soluble amount<br>compared to H3κ2 | κ1   | κ2   | κ3   | κ4   | λ1   | λ2   | λ3   |
|------------------------------------|------|------|------|------|------|------|------|
| H1A                                | 191% | 88%  | 121% | 122% | 26%  | 211% | 76%  |
| H1B                                | 124% | 95%  | 83%  | 107% | 79%  | 142% | 59%  |
| H2                                 | 126% | 204% | 139% | 130% | 66%  | 50%  | 70%  |
| H3                                 | 63%  | -    | 81%  | 49%  | 69%  | 143% | 61%  |
| H4                                 | 40%  | 47%  | 49%  | 54%  | 95%  | 55%  | 125% |
| H5                                 | 69%  | 158% | 116% | 80%  | 72%  | 84%  | 84%  |
| H6                                 | 85%  | 122% | 87%  | 77%  | 162% | 162% | 212% |
|                                    | McPC |      |      |      |      |      |      |
| soluble                            | 38%  |      |      |      |      |      |      |
| %H3κ2 total                        | 117% |      |      |      |      |      |      |
| %H3κ2 soluble                      | 69%  |      |      |      |      |      |      |

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 96/03647

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/13 C12N15/10 C12N15/62 C12N15/70 C12N1/21  
C07K1/04 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                       | Relevant to claim No. |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | EP 0 368 684 A (MEDICAL RES COUNCIL) 16<br>May 1990<br>cited in the application<br>see the whole document<br>---                                                                                                                                                                                         | 1-55                  |
| A          | EUROPEAN J. IMMUNOLOGY,<br>vol. 23, July 1993, VCH<br>VERLAGSGESELLSCHAFT MBH, WEINHEIM, BRD,<br>pages 1456-1461, XP000616572<br>S.C. WILLIAMS AND G. WINTER: "Cloning and<br>sequencing of human immunoglobulin<br>V-lambda gene segments"<br>cited in the application<br>see the whole document<br>--- | 1-55                  |

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of mailing of the international search report

11.02.97

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Fax: (+31-70) 340-3016

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|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | <p>PROC. NATL.ACAD SCI.,<br/>vol. 89, May 1992, NATL. ACAD<br/>SCI.,WASHINGTON,DC,US;,<br/>pages 4457-4461, XP002024223<br/>C. F. BARBAS III ET AL.: "Semisynthetic<br/>combinatorial antibody libraries: a<br/>chemical solution to the diversity<br/>problem"<br/>cited in the application<br/>see the whole document<br/>---</p> | 1-55                  |
| A          | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF<br/>SCIENCES OF USA,<br/>vol. 89, no. 21, 1 November 1992,<br/>pages 10026-10030, XP000322464<br/>COLLET T A ET AL: "A BINARY PLASMID<br/>SYSTEM FOR SHUFFLING COMBINATORIAL<br/>ANTIBODY LIBRARIES"<br/>see the whole document<br/>---</p>                                               | 1-55                  |
| A          | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF<br/>SCIENCES OF USA,<br/>vol. 89, no. 8, 15 April 1992,<br/>pages 3576-3580, XP000384398<br/>GRAM H ET AL: "IN VITRO SELECTION AND<br/>AFFINITY MATURATION OF ANTIBODIES FROM A<br/>NAIVE COMBINATORIAL IMMUNOGLOBULIN<br/>LIBRARY"<br/>see the whole document<br/>---</p>                | 1-55                  |
| A          | <p>PROTEIN ENGINEERING,<br/>vol. 8, no. 1, 1 January 1995,<br/>pages 81-89, XP000500393<br/>KNAPPIK A ET AL: "ENGINEERED TURNS OF<br/>RECOMBINANT ANTIBODY IMPROVE ITS IN VIVO<br/>FOLDING"<br/>cited in the application<br/>see the whole document<br/>---</p>                                                                     | 1-55                  |
| A          | <p>ANNUAL REVIEW OF IMMUNOLOGY,<br/>vol. 12, 1 January 1994,<br/>pages 433-455, XP000564245<br/>WINTER G ET AL: "MAKING ANTIBODIES BY<br/>PHAGE DISPLAY TECHNOLOGY"<br/>cited in the application<br/>see the whole document<br/>---</p>                                                                                             | 1-55                  |
| A          | <p>JOURNAL OF MOLECULAR BIOLOGY,<br/>vol. 224, no. 2, 1 January 1992,<br/>pages 487-499, XP000564649<br/>FOOTE J ET AL: "ANTIBODY FRAMEWORK<br/>RESIDUES AFFECTING THE CONFORMATION OF THE<br/>HYPERCARIABLE LOOPS"<br/>cited in the application<br/>see the whole document<br/>---</p>                                             | 1-55                  |

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## INTERNATIONAL SEARCH REPORT

Int ional Application No  
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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                       |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category *                                           | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                | Relevant to claim No. |
| A                                                    | <p>NUCLEIC ACIDS RESEARCH,<br/>vol. 21, no. 9, 11 May 1993,<br/>page 2265/2266 XP000575849<br/>WATERHOUSE P ET AL: "COMBINATORIAL<br/>INFECTION AND IN VIVO RECOMBINATION: A<br/>STRATEGY FOR MAKING LARGE PHAGE ANTIBODY<br/>REPERTOIRES"<br/>see the whole document</p> <p>---</p>                                                                                                                                                              | 1-55                  |
| A                                                    | <p>WO 95 11998 A (UNITED BIOMEDICAL INC) 4<br/>May 1995<br/>see the whole document</p> <p>---</p>                                                                                                                                                                                                                                                                                                                                                 | 1-55                  |
| A                                                    | <p>ANNALES DE BIOLOGIE CLINIQUE,<br/>vol. 49, no. 4, April 1991, PARIS, FR,<br/>pages 231-242, XP000407361<br/>R.H. MELOEN ET AL.: "The use of peptides<br/>to reconstruct conformational<br/>determinants"<br/>see page 231, right-hand column, paragraph<br/>2 - page 233, right-hand column, line 4</p> <p>---</p>                                                                                                                             | 1-55                  |
| A                                                    | <p>CHEMICAL ABSTRACTS, vol. 122, no. 3,<br/>16 January 1995<br/>Columbus, Ohio, US;<br/>abstract no. 24865z,<br/>COX, JONATHAN P. L. ET AL: "A directory<br/>of human germ-line V.kappa. segments<br/>reveals a strong bias in their usage"<br/>page 227; column 1;<br/>XP0002024224<br/>cited in the application<br/>see abstract<br/>&amp; EUR. J. IMMUNOL. (1994), 24(4), 827-36<br/>CODEN: EJIMAF;ISSN: 0014-2980,<br/>1994,</p> <p>-----</p> | 1-55                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| EP-A-0368684                              | 16-05-90            | AU-B- 634186               | 18-02-93            |
|                                           |                     | AU-A- 4520189              | 28-05-90            |
|                                           |                     | CA-A- 2002868              | 11-05-90            |
|                                           |                     | DE-D- 68913658             | 14-04-94            |
|                                           |                     | DE-T- 68913658             | 08-09-94            |
|                                           |                     | ES-T- 2052027              | 01-07-94            |
|                                           |                     | WO-A- 9005144              | 17-05-90            |
|                                           |                     | JP-T- 3502801              | 27-06-91            |
| -----                                     |                     |                            |                     |
| WO-A-9511998                              | 04-05-95            | AU-A- 8091694              | 22-05-95            |
|                                           |                     | EP-A- 0725838              | 14-08-96            |
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